

**THE ECOLOGY AND EVOLUTION OF INDUCIBLE DEFENSES IN THE
FRESHWATER SNAIL *HELISOMA TRIVOLVIS***

by

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The effects of environmental variation on the phenotypes expressed by organisms have gradually gained interest in biology. This interest has been sparked by the realization that environment-dependent phenotypic expression (i.e. phenotype plasticity) can improve an organism's fitness when different environments are encountered. The challenge for researchers is to determine the importance of phenotypic plasticity to the various fields of biology.

A major goal in community ecology is to understand predator-prey interactions within natural communities. Recently, ecologists have focused their attention towards the inducible defenses of prey with the realization that prey are not simply passive participants but instead express a variety of inducible defenses. This dissertation explores the ecology and evolution of inducible defenses using freshwater snails and their predators as a model system. My specific objectives were to identify the adaptive significance of induced responses against predators, to explore the importance of development for understanding inducible defenses, and to address the phenotypic and fitness consequences of spatial and temporal variation in predation risk on prey species.

Snails were extremely flexible in their ability to respond to different predator environments. It was evident in each of my experiments that snails were able to alter a unique suite of traits with different predators and integrate their phenotypic responses to environments that contained multiple predator species. Moreover, phenotypic trade-offs resulting from internal resource competition among traits appear to be the underlying mechanism for the expression of inducible defenses. I also found induced defenses can come at the cost of reduced growth, delayed reproduction, or reduced fecundity. However, these costs were dependent on the identity of the predator. By incorporating a developmental perspective, I was able to document that snails have wide developmental windows for formation of defenses but narrow windows for

the reversal of defenses. Moreover, the lag time associated with the formation of some defenses can limit the benefits of the defense. Lastly, responses to predators early in development can constrain future responses to different predators and have dramatic impacts on fitness.

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PREFACE

I begin by thanking my advisor, Dr. Rick Relyea. In 1999, you hired a wide-eyed undergraduate with a love for herps but little experience. In 2000, you encouraged a hesitant laboratory technician to apply for graduate school to pursue his scientific interests. In 2007, that same person thanks you for everything that you have done. You have been a fantastic mentor. Your ability to teach the basics of our field while demonstrating how to push the field to the frontier has been astonishing. Your insights on life as an academic have also been invaluable for preparing me for my future in science. I can only hope that my use of your model with future students is as successful. I would also like to acknowledge that through out this dissertation I use the personal pronoun “I” but this work was conducted in collaboration with Rick.

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1.0 INTRODUCTION

Because of the complexity of natural systems, biological research has radiated into a variety of different fields including development, genetics, ecology, and evolution. While these fields have diverged over the years addressing unique questions at different levels of biological organization, one of the major factors keeping the fields united is phenotypic variation. Indeed, phenotypic variation is what natural selection has acted on to generate the biological diversity that has astounded and inspired scientists for generations. Consequently, our past goals as biologists are parallel to our current goals, identify what generates phenotypic variation and determine the consequences of that variation.

In the last half of the 20th century, it became clear that environmental variation could have dramatic effects on the phenotypes of nearly all organisms (Schmalhausen 1949, Waddington 1953, Bradshaw 1965). Moreover, for organisms that experience environmental variation, a single phenotype is typically not optimal across all environments. However, if there are reliable cues of environmental change, organisms can alter their phenotypic expression to improve their fitness. Phenotypic plasticity, the ability of a single genotype to produce different phenotypes under varying environmental conditions, has been growing at a tremendous rate as biologists appreciate that a great deal of the phenotypic variation observed in nature is the result of adaptive responses to environmental heterogeneity and not simply uninteresting “noise” (Schlichting and Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003). Recently, the challenge for researchers has been to integrate phenotypic plasticity into the various fields of biology to determine the importance of this new perspective for understanding phenotypic variation.

Predator-prey systems have made major contributions to our understanding of phenotypic plasticity (i.e. inducible defenses). Within natural communities, prey encounter temporal and spatial variation in predation risk and they use a variety of visual, tactile and chemical cues to evaluate this risk (Lima and Dill 1990, Chivers and Smith 1998, Kats and Dill 1998). In

response to predator cues, prey express a diversity of phenotypic changes in behavior, life history, and morphology (Tollrian and Harvell 1999). These responses can lead to lower predation rates but typically come at the cost of slower growth, development, or fecundity. Together, this research has shown that inducible defenses can be a viable strategy for reducing the risk of predation. Thus, organisms with inducible defenses provide excellent model systems to explore the numerous questions that remain unexplored concerning the ecology and evolution of phenotypic plasticity.

Several authors have argued that to understand the complexity of adaptive phenotypic plasticity we must take a comprehensive approach that includes multiple environments (>2), multiple traits, and several ontogenetic stages (Schlichting and Pigliucci 1998, Pigliucci 2001, Van Buskirk 2002, DeWitt and Langerhans 2003, Ghalambor et al. 2003, West-Eberhard 2003, Relyea 2004). With a more comprehensive approach, we can identify functional relationships between traits, constraints on phenotypic responses, and the cost and benefits of alternative phenotypes. In predator-prey systems, ecologists have documented how a diversity of prey respond to predators, but few studies have conducted in-depth experiments that investigate how prey integrate a variety of traits (e.g., behavior, morphology, and life history) in response to a wide range of environments. This concern is addressed in chapter 2 (a manuscript that has been published in Oecologia; Hoverman et al. 2005).

Inducible defenses allow prey to modulate their phenotypic responses to the level of predation risk in the environment and reduce the cost of constitutive defenses. However, we often overlook the importance of development in controlling developmental trajectories of traits, integrating phenotypes over ontogeny, and establishing developmental windows of trait formation and reversal (Diggle 1994, Novoplansky et al. 1994, Gabriel 1999, Gilbert 2001, West-Eberhard 2003). By moving away from studies that focus on a single point in development, we can obtain a more complete understanding of the phenotypic decisions and limitations of prey. I assess the role of development in understanding inducible defenses in chapter 3 (a manuscript that has been published in Ecology; Hoverman and Relyea 2007).

Prey rarely encounter a single predator in natural communities but coexist with a diversity of predator species that often differ functionally (Sih et al. 1998, Chalcraft and Reserits 2003). Prey that encounter combinations of predators must make phenotypic decisions that account for the relative risk of each predator, the frequency of encountering each predator,

and the effectiveness of the defenses (Lima 1992; Matsuda et al. 1993, Peckarsky and McIntosh 1998, McIntosh and Peckarsky 1999, Turner et al. 2000, Relyea 2003, Wiackowski et al. 2003). By examining different predator combinations and multiple prey traits, ecologists can obtain an excellent understanding of how prey respond to more complex predator regimes. Moreover, we can generate predictions about prey survival with combined predators by combining data on the non-lethal effects of combined predators on prey defenses and an understanding of the fitness trade-offs associated with different phenotypes. I address these ideas in chapter 4 (a manuscript that has been submitted to Oecologia; Hoverman and Relyea *in review a*).

During the past decade, research on inducible defenses has demonstrated that induced phenotypes are of importance not only to the individual that changes but also to the larger ecological community via trait-mediated indirect interactions (TMIIs; Abrams 1995, Werner and Peacor 2003, Schmitz et al. 2004). However, much of this work has focused on predator risk that is variable in space. Prey within natural communities can experience substantial temporal variation in predation risk that poses unique challenges on the defensive strategies of prey. In chapter 5, I examine how spatial and temporal variation in predation regime via predator identity and predator colonization affect the phenotypes expressed by prey and the subsequent TMIIs in a community (a manuscript that has been submitted to Oikos; Hoverman and Relyea *in review b*).

This dissertation contributes to our understanding of phenotypic plasticity in a number of ways. First, my research has shown that there are a number of costs associated with the induced phenotypes. I demonstrated that phenotypic plasticity can lead to delayed reproduction and reduced fecundity (chapter 2). However, the magnitude of these costs (e.g., fecundity) were not equal among the environments and could be alleviated by other factors such as resource levels. Ecological costs were also prominent in my work. More specifically, the induced phenotypes against one predator generally increased susceptibility to other functional different predators (chapter 5). I also identified a number of limitations to the expression of phenotypic plasticity (i.e. developmental windows and epiphenotypes; chapter 3). Together the costs and limits of phenotypic plasticity are important factors affecting the evolution of adaptive plasticity. My work shows that organisms, which experience a wide variety of environments, have remarkable sensory systems for detecting environmental change. Indeed, prey are able to distinguish among predator species, determine predator abundance (i.e. density of predators), and assess the diet of the predator. This information is integrated to fine-tune the phenotypic responses of prey to the

‘perceived’ level of predation risk in the environment. Importantly, this information moves us towards understanding how prey may respond in natural communities where predation cues are highly variable. Lastly, I showed that temporal not only affects the individual, but also can affect the larger ecological community via trait-mediated indirect effects. In sum, this dissertation demonstrates the effects of environmental variation on prey phenotypes as well as the consequences of those responses at the individual and community levels.

2.0 PUTTING PREY BACK TOGETHER AGAIN: INTEGRATING PREDATOR-INDUCED BEHAVIOR, MORPHOLOGY, AND LIFE HISTORY

2.1 ABSTRACT

The last decade has seen an explosion in the number of studies exploring predator-induced plasticity. Recently, there has been a call for more comprehensive approaches that can identify functional relationships between traits, constraints on phenotypic responses, and the cost and benefits of alternative phenotypes. In this study, I exposed *Helisoma trivolvis*, a freshwater snail, to a factorial combination of three resource levels and five predator environments (no predator, 1 or 2 water bugs, and 1 or 2 crayfish) and examined 10 traits including behavior, morphology, and life history. Each predator induced a unique suite of behavioral and morphological responses. Snails increased near-surface habitat use with crayfish but not with water bugs. Further, crayfish induced narrow and high shells whereas water bugs induced wide shells and wide apertures. In terms of life history, both predators induced delayed reproduction and greater mass at reproduction. However, crayfish induced a greater delay in reproduction that resulted in reduced fecundity whereas water bugs did not induce differences in fecundity. Resource levels impacted the morphology of *Helisoma*; snails reared with greater resource levels produced higher shells, narrower shells, and wider apertures. Resource levels also impacted snail life history; lower resources caused longer times to reproduction and reduced fecundity. Based on an analysis of phenotypic correlations, the morphological responses to each predator most likely represent phenotypic trade-offs. Snails could either produce invasion-resistant shells for defense against water bugs or crush-resistant shells for defense against crayfish, but not both. My use of a comprehensive approach to examine the responses of *Helisoma* has provided important information regarding the complexity of phenotypic responses to different environments, the

patterns of phenotypic integration across environments, and the potential costs and benefits associated with plastic traits.

2.2 INTRODUCTION

Nearly every organism is phenotypically plastic for some trait (Travis 1994) and many environmentally-induced phenotypic changes are adaptive strategies that permit organisms to improve their fitness (Pigliucci 2001). Such phenotypic plasticity has received a great deal of attention from ecologists and evolutionary biologists because it allows organisms to possess a wide range of ecological options (Clausen et al. 1948, Bradshaw 1965, Cook and Johnson 1968, Schlichting 1986, Sultan 1987, West-Eberhard 1989, Schlichting and Pigliucci 1998). While numerous types of plasticity exist, one of the most studied is predator-induced plasticity (Karban and Baldwin 1997, Tollrian and Harvell 1999).

Empirical investigations of predator-induced plasticity have documented an extensive list of prey responses to a diverse array of predators (Dill 1987, Sih 1987, Kusch 1993, Warkentin 1995, Kats and Lima 1998, Tollrian and Harvell 1999, Pettersson et al. 2000, Van Buskirk 2002). Behavioral responses to predators are particularly well documented and include reduced activity, increased use of refuges, and spatial avoidance (Snyder 1967, Schmitt 1982, Dodson 1988, Holomuzki and Hoyle 1990, Abrahams and Healey 1993, Kusch 1993, Turner et al. 1999, Sih 2004). Prey can also form morphological defenses such as body shape changes (Brönmark and Miner 1992, Kuhlmann et al. 1999, Van Buskirk and Schmidt 2000) and the growth of defensive spines (Krueger and Dodson 1981, Harvell 1986, Havel and Dodson 1987) that reduce predation rates. In addition to behavior and morphology, some prey respond to predators by altering life-history strategies. Prey that can achieve a size refuge from predators frequently delay reproduction in favor of more rapid growth (Cowl and Covich 1990) whereas prey that become more vulnerable with increased size typically reproduce earlier or at a smaller size (Riessen 1999, Barry 2000, Johnson 2001). In short, we have an impressive body of work that has examined how different prey species alter their traits in response to predators.

While past studies have shown that prey can alter a variety of traits in response to different predators, several authors have argued that to understand the complexity of adaptive

phenotypic plasticity we must take a more comprehensive approach that includes more species, more environments (>2), a larger number of traits, and several ontogenetic stages (Schlichting and Pigliucci 1998, Van Buskirk 2002, DeWitt and Langerhans 2003, Pigliucci 2003, Ghalambor et al. 2003, West-Eberhard 2003, Relyea 2004). A major tool in applying this approach has been phenotypic integration (Olson and Miller 1958, Schlichting 1989, Wagner and Schwenk 2000, West-Eberhard 2003, Pigliucci and Preston 2004). While definitions of phenotypic integration vary, Pigliucci (2003) broadly defined it as the pattern of functional, developmental, and/or genetic correlation (however measured) among different traits. As the definition implies, phenotypic integration can be viewed from a variety of perspectives (e.g. development, genetics) and can provide information concerning the functional relationships between traits, changes in phenotypic strategies over ontogeny, constraints on phenotypic responses, and the cost and benefits of alternative phenotypes. In predator-prey systems, phenotypic integration has provided important contributions to understanding the complexity of prey phenotypic responses to their predators (Kuhlmann et al. 1999, Tollrian and Dodson 1999, DeWitt and Langerhans 2003, Relyea 2004). The challenge is to undertake a comprehensive approach that examines a large number of traits and environments to obtain a more complete understanding of phenotypic integration.

Freshwater snails are highly conducive to the execution of a comprehensive approach to predator-induced plasticity. Snails exhibit predator-induced behavior (Snyder 1967, Alexander and Covich 1991b, Turner et al. 1999, DeWitt et al. 1999, McCarthy and Fisher 2000), morphology (Appleton and Palmer 1988, Trussell 1996, DeWitt 1998, DeWitt and Langerhans 2003), and life history (Crowl and Covich 1990, DeWitt 1998, Chase 1999). Additionally, snails have been utilized to address the functional relationship between traits (DeWitt et al. 1999), the effects of multiple predators (DeWitt and Langerhans 2003), and trade-offs between growth and reproduction (Chase 1999).

In this study, I reared the freshwater snail *Helisoma trivolvis* (hereafter referred to as *Helisoma*) under different food rations and predator environments (water bugs, *Belostoma flumineum*; and crayfish, *Orconectes rusticus*) to understand how prey alter their suites of defensive traits (behavioral, morphological, and life history) in a variety of predator and resource environments. Water bugs and crayfish were chosen because they use contrasting habitats and feeding strategies that may favor alternative phenotypes in the system. Crayfish are restricted to

pond bottoms and consume *Helisoma* by shell chipping and crushing (J. T. Hoverman and R. A. Relyea, *unpublished data*). Therefore, crayfish should induce *Helisoma* to move higher in the water column and produce shells that are crush-resistant. In contrast, water bugs feed throughout the water column and consume snails via shell-invasion (Kesler and Munns 1989). Thus, water bugs should have no impact on *Helisoma* behavior (water bugs can search the entire water column) but induce the formation of invasion-resistant shells (i.e. shells that prevent water bugs from reaching the interior snail body). These hypothesized morphological responses to each predator likely represent trade-offs; snails cannot simultaneously produce shells that are both crush-resistant and invasion-resistant. In contrast to behavior and morphology, I expect the two predators to induce similar life history responses. Because predation rates decline as snail size increases (Alexander and Covich 1991a, Chase 1999), I expect *Helisoma* to delay reproduction and reach a larger size at reproduction at the cost of reduced fecundity (Tsitrone et al. 2003).

I tested the following hypotheses: 1) water bugs will not induce habitat shifts but crayfish will induce *Helisoma* to move to the surface; 2) water bugs and crayfish will induce opposite morphological defenses; 3) water bugs and crayfish will induce similar life-history responses; 4) snails will produce more extreme phenotypes with increased predator densities; and 5) decreased food rations will cause weaker anti-predator responses, longer times to reproduction, smaller sizes at reproduction, and lower egg production.

2.3 METHODS

Adult *Helisoma* were collected on 1 September 2002 from Geneva Pond #1, a semi-permanent pond that contains water bugs but no crayfish or fish (located in northwestern Pennsylvania). In the laboratory, the adults were placed into 10-L plastic tubs filled with 7-L of carbon-filtered, UV-irradiated tap water to allow oviposition. Egg masses were laid over a span of 2 wks and hatching began on 21 September 2002. Hatchlings were fed ground *Spirulina* fish food (OSI Marine Inc., California) *ad lib.* until the start of the experiment. Hatchlings were raised for 4 wks until they were large enough to be transferred into the experimental containers.

The experiment began on 22 October 2002 using a randomized block design with a factorial combination of three resource levels and five predator treatments. The three resource

levels were rations of *Spirulina* at 2.5%, 5%, and 10% of mean snail mass per day. From previous experiments with *Helisoma*, a 10% food ration was found to be sufficient for normal growth and development (J. T. Hoverman and R. A. Relyea, *unpublished data*). Food rations were doubled when the snails doubled in biomass (averaged across groups). The five predator treatments were the following: no predator, one caged water bug, two caged water bugs, one caged crayfish, or two caged crayfish. The experimental blocks were four sets of shelves that differed in height (and temperature). Thus, the 15 treatment combinations were replicated four times (one replicate per block) for a total of 60 experimental units. The experimental units were 10-L plastic tubs filled with 7-L of carbon-filtered, UV-irradiated tap water. Ten snails were added to each tub (mean mass \pm 1 SE = 11 ± 2 mg). Twenty snails were set aside to assess 24-hr survival after handling; survival was 100%. Snails were fed three times per week and the water was changed once per week to prevent fouling. The animal rearing room was held at 21°C and a 14:10 hr day:night cycle.

Each tub was equipped with two predator cages constructed of a 180-ml plastic cup covered with a screen. Cages remained empty in tubs assigned the no-predator treatment. In tubs assigned predator treatments, either one or both cages held an individual predator (either a water bug or a crayfish). Water bugs were collected from several ponds near Geneva Marsh whereas crayfish were collected from Linesville Creek. *Helisoma* commonly coexist with these two predators in lakes, marshes, and ponds in western Pennsylvania (J. T. Hoverman, *personal observation*). Each predator was fed approximately 120 mg of snails three times per week. Empty cages were briefly lifted out of the water and then returned to the tubs to equalize disturbance among all tubs. Dead snails were removed from predator cages.

Late in the experiment (18 January 2003), several crayfish died and replacement crayfish were not available (due to the frozen conditions of their native habitat). Thus, I changed my method of producing predatory cues in the water. I removed all predators from the tubs and placed them in several separate tubs of water to serve as sources of predatory cues (one “cue tub” for each treatment). The cue tubs contained 4-L of water and either one or two predators were housed in cages and fed 120 mg of snail biomass three times per week. Each day, I transferred water from these cue tubs to the appropriate experimental tubs. For treatments assigned one predator, I added 60 ml of cue water from tubs with one predator; for treatments assigned two predators, I added 60 ml of cue water from tubs with two predators. For no-predator tanks, I

added 60 ml of aged tap water. This later cue-tub protocol was approximately 10% of the cue concentration of the original caged predator design. However, reducing the strength of the predator cue late in the experiment would simply bias against detecting a predator effect.

To quantify snail behavior, I made visual observations using scan sampling (Altmann 1974) on 18 January 2003. I recorded the percentage of snails that were within 3 cm of the water's surface, a commonly-used measure of habitat choice in snails (Turner 1996, DeWitt 1998). I made 10 observations on each tub and used the mean proportion from each tub as my response variable. In two tubs, the predators were dead on the day of observation. Thus, I excluded these tubs from my analysis.

To quantify snail life-history traits, I examined the time to reproduction, mass at reproduction, and the total number of eggs produced per snail. I defined time to reproduction as the number of days until the first egg mass appeared in a tub. I defined mass at reproduction for a tub as the average mass of the snails when the first egg mass appeared in a tub. The first egg masses were laid on 27 December 2002. By late February, most snail treatments contained eggs (except under the lowest food treatment). During this time (typically three times per week), I counted the number of eggs in each egg mass and the number of adult snails alive in the tub. After the masses were counted, I removed the masses from the tubs. I standardized the egg counts by dividing the values by the number of adult snails alive in each tub. I then used the standardized counts to calculate my response variables (the total number of eggs per snail). For all response variables, I used tub means.

The experiment was terminated after 4 months (21 February 2003). All snails were preserved in 10% formalin. Preserved snails were subsequently blotted dry, weighed for final mass, and measured using digital imaging software (Optimas Co., Bothell, WA). With the snails resting on their umbilical side, I measured shell width and aperture width (Fig. 2.1). With the snails resting with their aperture up, I measured, shell height, aperture height and shell thickness. Shell thickness was measured at the leading edge of the aperture.

2.3.1 Statistical analyses

In the analysis of morphological plasticity, I was interested in how shape changes independent of changes in overall size. I used analysis of covariance (ANCOVA) with mass as the covariate to

correct for size (Darlington and Smulders 2001, Garcia-Berthou 2001). All traits were log transformed prior to analysis to improve the linearity of the relationship. A critical assumption in the ANCOVA procedure is that the treatments share a common slope of their regression lines. This was satisfied for all the traits except shell thickness. Upon closer examination, I found no relationship between shell thickness and mass. Therefore, I used the raw measurement of thickness in my analysis. For the remaining variables, I used the mass-adjusted group mean and residuals from the within-group regression to calculate each individual's size-adjusted value. For each morphological trait, I then calculated the mean response for each experimental unit and used this as my morphological response variable.

The data were divided into two analyses. In the first analysis, I began by conducting a principal components analysis (PCA) on the single behavioral trait (near-surface habitat use), the five morphological traits, and final mass (see Table 2.1). The first two principal components had eigenvalues greater than 1 and, thus, they were extracted for the analysis. For each tub, I calculated the mean PC-1 and PC-2 scores and subjected these tub scores to univariate analysis of variance (ANOVA) to examine the effects of block, treatments, and their interactions. I conducted separate ANOVAs for PC-1 and PC-2 because these variables are uncorrelated by definition. Because two tubs were missing values for near-surface habitat use (due to dead predators on the day of observation), they were excluded from the analysis. In the ANOVAs, the main effect of block (i.e. shelf height) and block interactions were not significant and not retained in the model; thus, their degrees of freedom and sums-of-squares were pooled with the error term. Mean comparisons were conducted using the Fisher LSD test.

In the second analysis, I examined the effects of the treatments on snail life history (i.e. time to reproduction, mass at reproduction, egg production) using only the 5% and 10% food rations because snails fed the 2.5% ration did not reach reproduction in most tubs. I subjected the tub means to a PCA and only PC-1 had an eigenvalue greater than 1. Using the PC-1 scores for each tub, I used ANOVA to examine the effects of block, treatments, and their interactions. Block effects and their interactions were never significant; therefore, their degrees of freedom and sums-of-squares were pooled with the error term.

Although PCA can reveal overall patterns in multivariate data, some traits may not follow the general pattern of a particular principle component. In both analyses, most traits were well represented in the patterns depicted by the principle component scores (as indicated by

communality values > 0.6 ; McGarigal et al. 2000). However, both analysis presented some problems when interpreting the results. For five of the 10 traits used in the study, the pattern of treatment means on the principal components did not adequately describe the treatment effect for the variables. Therefore, I conducted additional univariate ANOVAs on these traits. My goal with this approach was to provide the reader with the most accurate picture of how all traits were affected by the treatments.

2.3.2 Trait correlations

I used correlation analysis to examine the degree of trait integration associated with *Helisoma*'s responses to the treatments and to provide insights into possible phenotypic trade-offs across environments (Via and Lande 1985, 1987; Schlichting 1989). I constructed a Pearson correlation matrix of the 10 traits across the 15 treatments. Because some tubs lacked reproductive data or behavioral data, the number of experimental units per trait ranged from 48 to 60. A Bonferroni correction was made for conducting the 45 possible correlations.

2.4 RESULTS

The first analysis started by conducting a PCA on snail behavior, morphology, and final mass. The first two principal components accounted for 68% of the variance in the data. PC-1 had an eigenvalue of 2.77 and accounted for 40% of the variance. Positive loadings on PC-1 were related to narrow and high shells, more use of surface habitats, and large final mass (Table 2.1, Fig. 2.2). PC-2 had an eigenvalue of 1.98 and accounted for 28% of the variance. Positive loadings on PC-2 were related to high apertures, wide apertures, and thick shells (Table 2.1, Fig. 2.2). I also examined final communality (how well the original variables were represented by the retained principal components; McGarigal et al. 2000). Most variables exhibited good agreement (communalities > 0.6). However, near-surface habitat use, shell thickness, and final mass had relatively low values (0.48 to 0.59), suggesting that the retained principal components did not exhibit excellent agreement with these variables.

Using the scores for PC-1, I conducted a ANOVA to determine the effects of treatments and their interactions. There was a significant effect of predators ($F_{4,43} = 32.8$, $P < 0.0001$) and food ($F_{2,43} = 9.4$, $P > 0.0001$) but no predator-by-food interaction ($F_{8,43} = 0.5$, $P = 0.489$). Based on mean comparisons, snails reared at the 2.5% food ration had marginally lower scores (i.e. wider and lower shells, less use of surface habitats, and smaller final mass) compared to snails reared at 5% ($P = 0.065$). Both the 2.5% and 5% food rations had smaller scores compared to snails reared at 10% ($P \leq 0.016$). Among the predator treatments, snails reared with crayfish had higher scores (i.e. narrower and higher shells, more use of surface habitats, and larger final mass) than snails reared without predators ($P \leq 0.0001$). While, snails reared with water bugs had similar scores (one water bug, $P = 0.107$) or lower scores (two water bugs, $P = 0.021$) than snails reared without predators. Snails reared with crayfish had higher scores compared to snails reared with water bugs ($P < 0.0001$). Within each predator species, there were no differences between snails reared with one or two predators ($P \geq 0.453$).

Using the scores for PC-2, I conducted a second ANOVA to determine the effects treatments and their interactions. There were no significant effects of predators ($F_{4,43} = 2.2$, $P = 0.084$), food ($F_{2,43} = 3.0$, $P = 0.063$), or the predator-by-food interaction ($F_{8,43} = 0.5$, $P = 0.864$).

For most traits, the PCA and ANOVA analyses adequately described the shifts in snail traits. However, for three traits (near-surface habitat use, shell height, and aperture width), a more complete understanding of the responses to water bugs and crayfish was obtained by also examining the univariate ANOVAs. When I examined shell height and near-surface habitat use more closely, I confirmed that crayfish induced increases in both traits compared to the no-predator treatments ($P \leq 0.0001$), but I found that water bugs did not ($P \geq 0.358$). In addition, I found no food effect for habitat use ($P = 0.397$). Despite the lack of significant effects for PC-2, I detected significant univariate effects of predator and food treatments on aperture width ($P \leq 0.0001$ and $P = 0.033$, respectively). Snails reared with water bugs developed wider apertures than snails reared with no predators or crayfish ($P \leq 0.005$), but there was no difference between the no-predator and crayfish treatments ($P \geq 0.5$). Snails reared at the 10% food ration produced wider apertures than snails reared at 2.5% and 5% ($P \leq 0.05$) but there was not a difference between snails reared at 2.5% and 5% ($P = 0.569$).

In the second analysis, I examined snail life history responses (time to reproduction, mass at reproduction, and egg production) using only the 5% and 10% food rations. From the PCA,

only the first principal component was extracted (eigenvalue = 2.02) and it accounted for 68% of the variance in the data. Positive loadings on this component were related to long time to reproduction, large size at reproduction, and less egg production (PC loadings = 0.933, 0.673, and -0.837, respectively; communalities = 0.871, 0.453, and 0.700, respectively).

The ANOVA on this life history principle component (PC-1) found significant effects of predators ($F_{4,27} = 8.9$, $P < 0.001$) and food ration ($F_{1,27} = 28.0$, $P < 0.001$) but no predator-by-food interaction ($F_{4,27} = 2.0$, $P = 0.122$). Snails reared at higher food rations reproduced earlier, at a smaller size, and produced more eggs (i.e. lower scores; Fig. 2.3). Based on mean comparisons, snails reared with crayfish and water bugs reproduced later, at a larger size, and produced fewer eggs (i.e. higher scores) than snails reared without predators ($P \geq 0.036$). Snails reared with one water bug had lower scores than snails reared with one or two crayfish ($P \leq 0.050$). Snails reared with two water bugs were not different from snails reared with two crayfish ($P = 0.153$) but had lower scores than snails reared with one crayfish ($P = 0.013$). Within each predator species, there were no differences between snails reared with one or two predators ($P > 0.26$).

In this analysis, mass at reproduction had a low communality value (0.45). Once again, I felt that a more complete understanding of the responses to water bugs and crayfish was obtained by also examining the univariate ANOVA for this trait. Predator treatments had a significant univariate effect on mass at reproduction ($P \leq 0.0001$) while food ration ($P = 0.459$) and the predator-by-food interaction did not ($P = 0.111$). In agreement with the PCA, snails reared with water bugs and crayfish were larger at reproduction than snails reared without predators ($P \leq 0.002$). In slight contrast to the PCA, snails reared with water bugs were not different from snails reared with crayfish ($P \geq 0.166$). A more accurate understanding of egg production was also possible with an univariate ANOVA. Consistent with the PCA, snails reared with crayfish produced fewer eggs relative to snails reared without predators. However, I found no differences between snails reared with water bugs compared to snails reared without predators ($P \geq 0.768$). This suggests that the rate of egg deposition in snails reared with water bugs was greater than those reared without predators because there was less time to deposit the eggs (due to the delay in reproduction).

2.4.1 Trait correlations

The analysis of phenotypic correlations was conducted across all 15 treatments of the experiment (Fig. 2.4). *Helisoma* displayed 15 significant correlations out of 45 possible correlations (Bonferroni-corrected $P \leq 0.00512$) suggesting a high level of integration among morphology, behavior, and life history. Across environments, wide shells were associated with wide apertures, low shells, small final mass, less use of surface-habitat, and greater egg production. High shells across environments were associated with large final mass, high apertures, thick shells, and more use of the surface. Five of the 15 significant correlations were negative suggesting possible phenotypic trade-offs in the system. For example, high shells were associated with narrow shells, large final mass was associated with delayed reproduction, and delayed reproduction was associated with low egg production.

2.5 DISCUSSION

This study explored the ability of a prey species to use multiple traits and trait types (i.e. behavior, morphology, and life history) in response to a range of predators and resource levels. The results demonstrated that *Helisoma trivolvis* changed life history and morphology in response to resources and changed behavior, morphology, and life history in response to predators. Moreover, the particular suite of defenses was dependent on predator identity.

Recent studies have shown that prey are capable of simultaneously altering suites of traits in the presence of predators and these responses can be predator-specific (Tollrian and Dodson 1999, Relyea 2001a, DeWitt and Langerhans 2003, Ghalambor et al. 2003). In this study, I examined prey responses to two predators that induce unique suites of morphological and behavioral traits. For example, water bugs induced no change in habitat use whereas crayfish induced greater use of surface habitats. Further, water bugs induced wide shells whereas crayfish induced narrow and high shells. Interestingly, the predators induced slightly different life-history changes; compared to snails living without predators, crayfish induced delayed reproduction, larger size at reproduction, and fewer eggs whereas water bugs induced delayed

reproduction and larger size at reproduction but a similar number of eggs. Thus, *Helisoma* has the ability to alter large suites of traits that are predator-specific.

Behavioral responses to predators have been well-documented in many prey taxa (Sih 1987, Lima and Dill 1990, Tollrian and Harvell 1999). In snails, the most common anti-predator behavior is spatial avoidance (Snyder 1967, Alexander and Covich 1991a, Turner 1996, DeWitt et al. 1999, McCarthy and Fisher 2000, Rundle and Brönmark 2001). For instance, predators that feed in the water column (i.e. fish, insects, and turtles) typically induce snails to seek benthic refuges whereas predators that feed in the benthos (i.e. crayfish, lobsters, crabs, and starfish) typically induce snails to move toward the surface. Consistent with previous studies, I found that crayfish induced *Helisoma* to move toward the surface. The extensive work of Snyder (1967) demonstrated that although some snail species respond to water bugs by burrowing into substrates, *Helisoma trivolvis* does not exhibit this response. This is consistent with my observations. However, it is possible that other anti-predator behaviors may occur within more complex habitats (e.g., rock crevices, macrophytes, substrates).

Over the last two decades, numerous studies have documented predator-induced morphological defenses across a variety of aquatic taxa (Schmitt 1982, Gilbert and Stemberger 1984, Havel 1987, Appleton and Palmer 1988, Brönmark and Miner 1992, Trussell 1996, Van Buskirk et al. 1997, DeWitt 1998, DeWitt et al. 2000, Relyea and Werner 2000). In freshwater snails, our knowledge of predator-induced morphological plasticity has come solely from snails with a spiral morphology (DeWitt 1998, Krist 2002) that is produced by secreting whorls around a central body axis resulting in a spire (see Fig 10.2 in Brown 1991). Importantly, this study examined planorbid snails that have a different coiling pattern; they secrete shell material in a spiral with the whorls lying in a single plane (Fig. 1). Additionally, physids and planorbids differ in the shape of the aperture (i.e. the “generating curve” for the shell; oval versus round, respectively; Raup 1962). Given these differences in shell morphology, I might expect the function of predator-induced morphological responses in physids and planorbids to be quite different. I found that crayfish induced *Helisoma* to develop relatively narrow and high shells but no changes in aperture shape. These responses may reflect the mode of attack by crayfish. Snyder (1967) found that for crayfish consuming a variety of snail species, “the mode of attack is ‘patient’ chipping away at the shell with the mandibles until the soft parts are reached” (p. 93). For this species of crayfish (*O. rusticus*), I have found that predation on *Helisoma* is entirely via

shell chipping and crushing and never via shell entry (J. T. Hoverman and R. A. Relyea, *unpublished data*). Thus, a likely explanation for the observed responses is that *Helisoma* is attempting to increase the roundness of the entire shell, which would increase overall shell strength (Rundle and Brönmark 2001, DeWitt et al. 2000). In contrast, crayfish typically attack physids via shell entry and induce physids to produce more elongate shells and apertures that restrict shell entry (DeWitt et al. 2000, DeWitt and Langerhans 2003). Interestingly, physids and planorbids have similar responses to crayfish in that they have greater expansion parallel to the coiling axis versus perpendicular to the axis. However, the function of those responses may be extremely different; greater parallel expansion in physids produces an elongate invasion-resistant shell whereas in planorbids it produces a round crush-resistant shell. Clearly, additional work is necessary to address the functional morphology of *Helisoma* responses to crayfish.

In past studies of freshwater snails, fish and crayfish have received the majority of the empirical attention as predators since they are dominant in permanent waters. However, water bugs can be a significant source of snail mortality, especially in habitats lacking fish (Kesler and Munns 1989). Water bugs feed on snails by grasping the shell in their forelegs, plunging their stylet down into the aperture, and piercing the soft tissue of the snail. This shell-invading tactic is fundamentally different from the shell crushing/chipping tactics displayed by crayfish; thus, I expected the morphological defenses induced by water bugs to be fundamentally different from those induced by crayfish. *Helisoma* behaviorally responds to water bug predation by retracting the body into the shell away from the stylet. To enhance this defensive strategy, *Helisoma* should increase the distance that the water bug must reach inside the shell before contacting the body. In my study, water bugs induced relatively wide shells and apertures, which should increase this retraction distance. Preliminary work on predation rates by water bugs has shown that *Helisoma* reared with water bugs for 2 wks and then exposed to lethal water bugs suffered 37% predation whereas snails reared without predators suffered 63% predation (J. T. Hoverman and R. A. Relyea, *unpublished data*). Therefore, the changes in shell shape induced by water bugs appear to be adaptive defenses against water bugs. Because water bugs and crayfish generally induce opposite morphological defenses, the two predators may help maintain selection for plastic morphology in *Helisoma*.

Based on life-history theory, size-selective predation on small individuals should favor rapid growth and delayed reproduction whereas predation on large individuals should favor slow

growth and early reproduction (Stearns and Koella 1986, Roff 1992, Stearns 1992, Abrams and Rowe 1996). Empirical support for these predictions has come from a variety of taxa (fish, Reznick and Endler 1982; *Daphnia*, Spitze 1992; amphipods, Wellborn 1994; tadpoles, Warkentin 2000). In freshwater snails, crayfish and water bugs selectively consume small snail size classes (Alexander and Covich 1991b, Chase 1999). As a consequence, snails typically delay reproduction and allocate resources to growth in the presence of these predators (Crowl and Covich 1990, Chase 1999, DeWitt 1998). In this study, crayfish and water bugs induced longer times to reproduction and greater mass at reproduction. Interestingly, snails reared with water bugs had a greater rate of egg deposition than snails reared without predators (i.e. less time to produce the same number of eggs). In contrast, crayfish, which induced longer delays in reproduction than water bugs, laid fewer eggs. Reduced egg production may have simply occurred because I stopped the experiment before the completion of the reproductive period, thereby restricting the length of time possible for egg deposition. Over longer time periods, snails in the crayfish treatments might eventually equalize their fecundity as seen with the water bug treatments. Alternatively, snails in the crayfish treatments may have laid fewer eggs because they were allocating additional resources to egg size to permit offspring to more quickly reach a size refuge (Roff 1992, Stearns 1992, Oksanen and Lundberg 1995), form morphological defenses, or as a consequence of behavioral responses (DeWitt 1998). Predator induction of a longer time to reproduction and greater mass at reproduction has been documented in other snail studies (Crowl 1990, Crowl and Covich 1990, DeWitt 1998, Chase 1999), but data on egg production have not been presented (both Crowl and Covich (1990) and DeWitt (1998) collected egg data but did not formally present the data). The egg data not only suggest that crayfish-induced snails experience lower fecundity, but that these shifts in prey life-history traits (i.e. egg production) may have important long-term effects on snail population sizes.

2.5.1 Effects of predator density and resource levels

The magnitude of predation risk can be an important factor in the induction of prey phenotypes. Many prey species respond to the presence of increased predator density by producing more extreme phenotypes (Loose and Dawidowicz 1994, Kusch 1995, Relyea 2004b). In freshwater snails, several studies using large experimental venues (wading pools and 38-L aquaria) have

shown that the magnitude of snail defenses increases with predation risk (Turner 1997, McCarthy and Fisher 2000). In my study, increased predator density had little impact on *Helisoma* phenotypes. However, given the small size of the experimental units (10-L plastic tubs), it is likely that the water was saturated with chemical cues. Additional research with *Helisoma* must be conducted before definite conclusions can be drawn.

There is a growing appreciation that the anti-predator responses of prey can depend upon the amount of resources or competition (Petranks 1989, Werner and Anholt 1996, Relyea 2002, Weetman and Atkinson 2002, Relyea and Hoverman 2003, Relyea 2004b). I examined predator-induced responses of *Helisoma* at three different food rations (2.5%, 5%, and 10% of snail body mass). I found that greater resource levels resulted in larger snails that have relatively high and narrow shells and wide apertures. Consistent with previous studies that have either experimentally increased resources (Eisenberg 1970, Chase 1999) or observed snails living in mesotrophic versus eutrophic habitats (Eversole 1978, Brown 1985), I found that higher food rations also increased fecundity, decreased the time to reproduction, but did not affect the mass at reproduction. Contrary to my expectations and the work of Chase (1999), I detected no interaction between resource level and predator treatment on *Helisoma*'s behavior, morphology, or life history. The discrepancies between Chase (1999) and my study could be due to a variety of protocol differences including different experimental venues (laboratory vs. outdoor mesocosms), types of resource manipulation (food additions vs. nutrient additions), and measures of size (mass vs. shell size).

2.5.2 What have we learned by taking a comprehensive approach to predator-induced plasticity?

Several researchers have called for a more comprehensive approach to examining phenotypic plasticity because it allows a more detailed examination of prey responses (Schlichting and Pigliucci 1998, Pigliucci, DeWitt and Langerhans 2003, Ghalambor et al. 2003, West-Eberhard 2003, Relyea 2004). Prey typically experience different species of predators that have different feeding strategies and can favor different prey defenses. In addressing how prey respond to different predators, the primary focus has been on behavioral defenses (Sih 1987, Relyea 2003). Such an approach may overlook the importance of other trait changes that can be

deployed in place of, or in addition to, behavior (Relyea 2001a, 2001b). By conducting a more comprehensive study to characterize how prey alter a large number of traits, I was able to document the formation of predator-specific suites of responses. The next step in plasticity research will be to determine the relative importance of each of these responses in reducing predation rates and the functional relationships among different traits (e.g. trait compensation or complementation; DeWitt et al. 1999).

A more comprehensive approach can help us evaluate the costs and benefits of predator-induced responses. Embedded in the theory of plasticity evolution is the assumption that no single phenotype is optimal in all environments (Dudley and Schmitt 1996). Therefore, there should be costs associated with anti-predator responses. In both plants and animals, the cost of induced defenses is typically reduced growth or fecundity in predator-free environments (Karban and Baldwin 1997, Kats and Dill 1998, Tollrian and Harvell 1999, Relyea 2002a). In snails, morphological defenses reduce predation rates at the cost of slower growth (Appleton and Palmer 1988). I found that induced defenses against crayfish in *Helisoma* came at the cost of reduced fecundity and potentially a lower intrinsic rate of population increase (r). Thus, I was able to identify potential costs associated with crayfish-induced responses by including multiple life-history traits.

By examining the integration of the traits with correlation analyses, I can also assess the basis of phenotypic trade-offs across environments in the system. For instance, there were a large number of significant correlations among the morphological traits suggesting that *Helisoma* could produce shells that are either wide or high, but not wide *and* high. Thus, the morphological defenses induced by one predator (e.g., water bugs) may result in greater susceptibility to the other predator (e.g., crayfish). While this hypothesis clearly needs to be tested in my system, research on other snail species have confirmed this prediction. For example, crayfish and fish generally induce opposite behaviors (near-surface habitat use vs. refuge use) and morphology (elongate apertures vs. round aperture) in physid snails (DeWitt 1998, Turner et al. 1999, DeWitt et al. 2000, Turner et al. 2000) and snails that respond to one predator can become more susceptible to the other predator (DeWitt et al. 2000, DeWitt and Langerhans 2003). Possible constraints on shell shape have been discussed previously by Raup (1966) and Gould (1980) who demonstrated that extant snail species exist only in a small portion of the possible morphological space (but see Schlichting and Pigliucci (1998) for a critique). In

sum, future work that addresses the underlying causes of phenotypic correlations (e.g., genetic correlations) may provide us with important information to address phenotypic trade-offs and evolutionary responses to predator environments.

2.5.3 Conclusions

Phenotypic plasticity is an expanding field of ecological and evolutionary research and a recent emphasis on more comprehensive studies has inspired researchers to explore suites of trait changes when an organism is exposed to different environments. In this comprehensive approach to understanding *Helisoma* plasticity, I found that *Helisoma* can alter a wide range of traits and that the suite of responses is environment-specific. Such a comprehensive approach provides important information regarding the complexity of phenotypic responses to different environments, the patterns of phenotypic integration across environments, the potential costs and benefits associated with the phenotypic responses, and potential effects of predators on long-term population dynamics. In short, a more comprehensive approach can provide a more complete understanding of prey responses to predators.

Table 2.1 Principal component structure for the first two principal components from a PCA on the behavior, morphology, and final mass of *Helisoma trivolvis*. Final communalities are shown for each variable.

Variable	PC 1	PC 2	Final Communality
Near-surface habitat use	0.689	0.312	0.571
Shell depth	-0.768	0.152	0.613
Shell width	-0.837	0.392	0.855
Shell height	0.707	0.513	0.763
Shell thickness	0.185	0.674	0.488
Aperture height	0.092	0.858	0.745
Aperture width	-0.604	0.624	0.754
Final mass	0.544	0.075	0.301

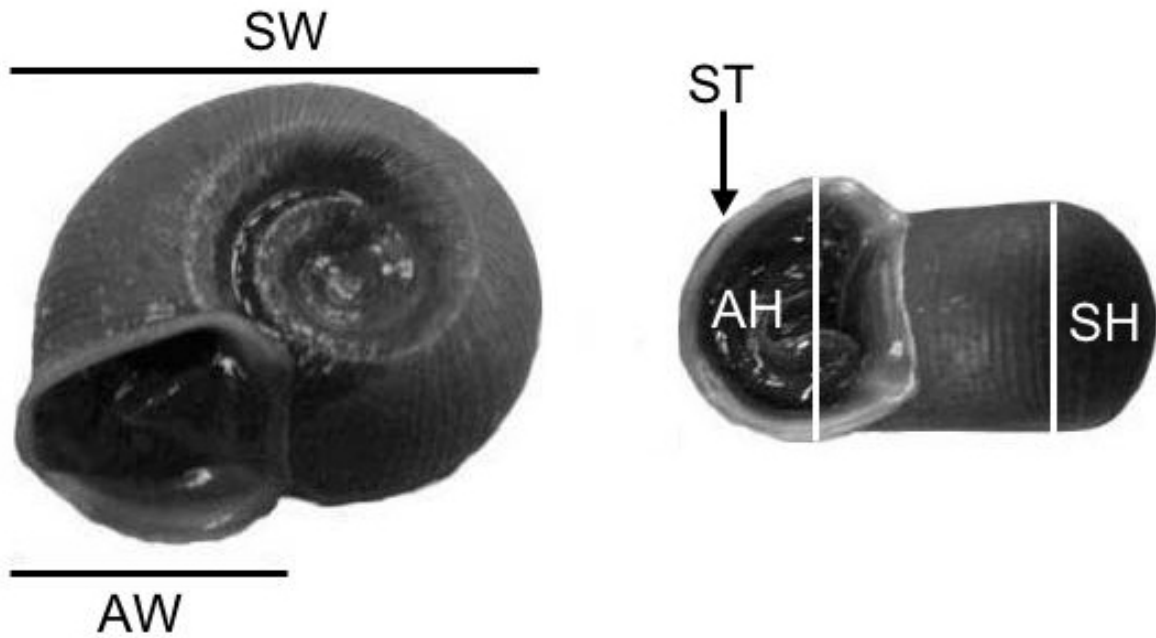


Figure 2.1 Morphological dimensions measured on *Helisoma trivolvis*. Abbreviations are as follows: SW = shell width, SH = shell height, ST = shell thickness, AW = aperture width, and AH = aperture height.

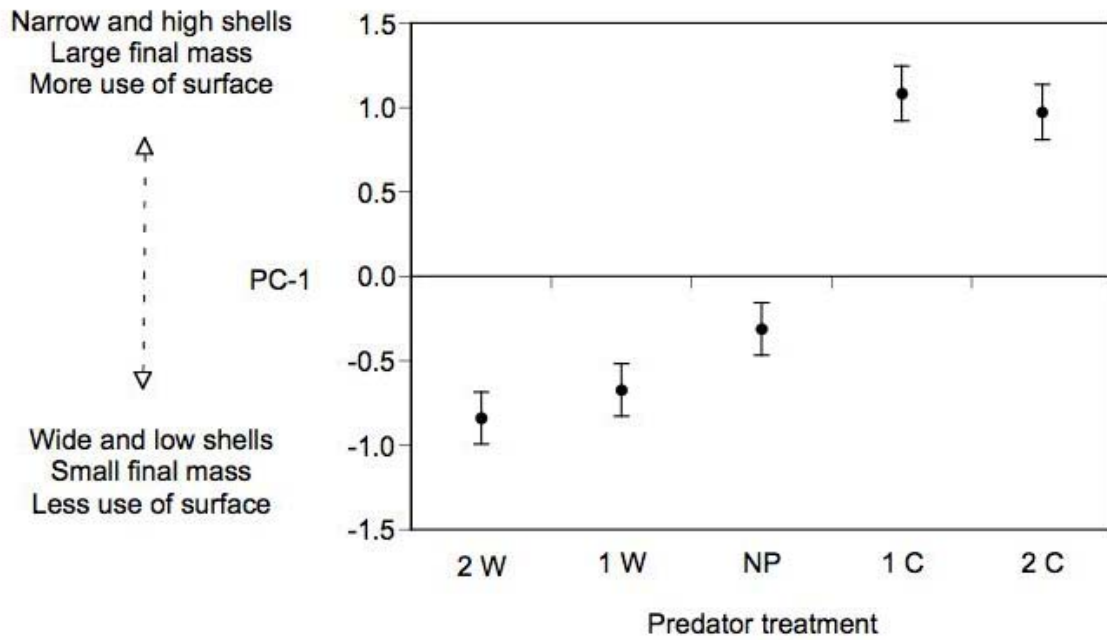


Figure 2.2 The effects of caged predators on principal component scores generated from a PCA on behavior, morphology, and final mass of *Helisoma trivolvis*. Treatments are abbreviated as follows: 2 W = 2 water bugs, 1 W = 1 water bug, NP = no predator, 1 C = 1 crayfish, 2 C = 2 crayfish. PC-1 accounted for 39.6% of the variation in the data set. Data are least-squares means \pm 1 SE.

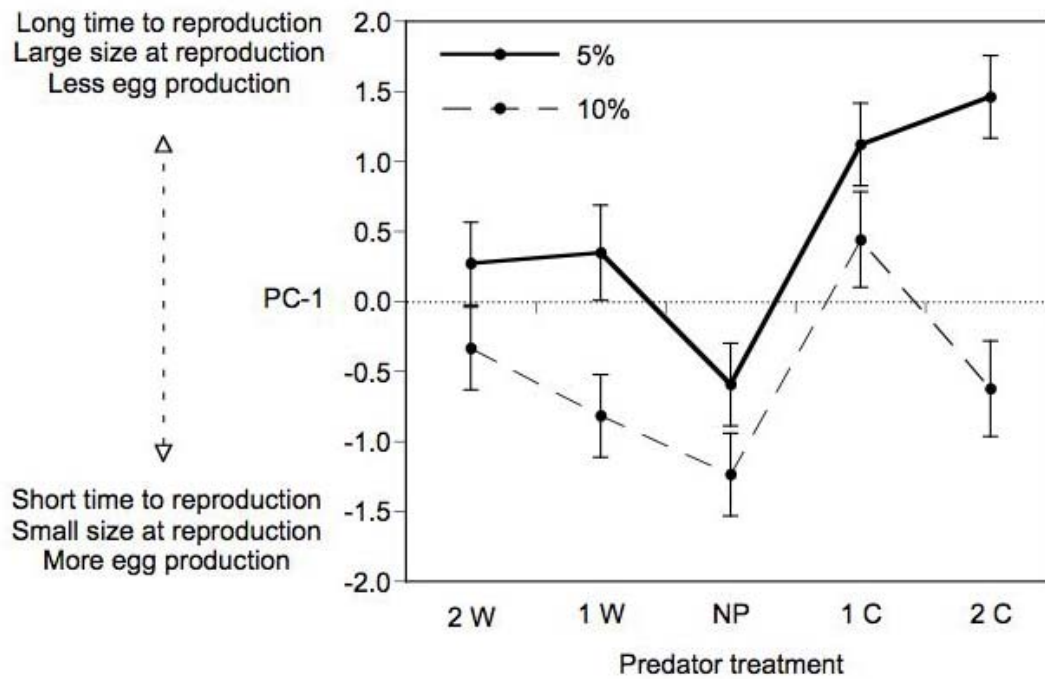


Figure 2.3 The effects of caged predators and food ration (Spirulina at 5% (—•—) or 10% (- • -) of snail's mass per day) on the first principal component generated from a PCA on *Helisoma trivolvis* life history (time to reproduction, size at reproduction, and egg production). Treatments are abbreviated as follows: 2 W = 2 water bugs, 1 W = 1 water bug, NP = no predator, 1 C = 1 crayfish, 2 C = 2 crayfish. PC-1 accounted for 67.5% of the variation in the data set. Data are least-squares means \pm 1 SE.

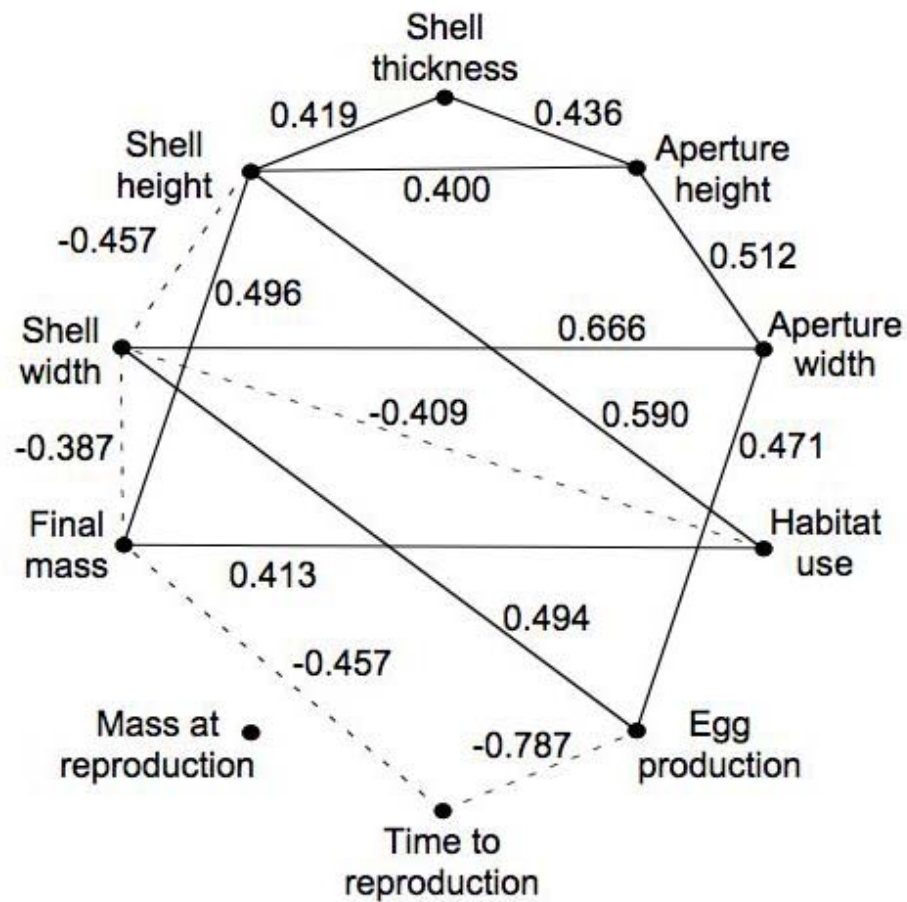


Figure 2.4 Trait correlations across predator treatments and food rations. All morphological responses are size-independent except shell thickness. Solid lines indicate positive trait correlations, whereas dashed lines indicate negative trait correlations. Only correlations with Bonferroni corrected $P \leq 0.00114$ are shown.

3.0 HOW FLEXIBLE IS PHENOTYPIC PLASTICITY? DEVELOPMENTAL WINDOWS FOR TRAIT INDUCTION AND REVERSAL

3.1 ABSTRACT

Inducible defenses allow prey to modulate their phenotypic responses to the level of predation risk in the environment and reduce the cost of constitutive defenses. Inherent in this statement is that prey must alter their phenotypes during development in order to form these defenses. This has lead many ecologists and evolutionary biologists to call for studies that examine developmental plasticity to provide insights into the importance of development in controlling the trajectories of trait formation, the integration of phenotypes over ontogeny, and the establishment of developmental windows for trait formation and reversal. By moving away from studies that focus on a single point in development, we can obtain a more complete understanding of the phenotypic decisions and limitations of prey. I exposed freshwater snails (*Helisoma trivolvis*) to environments in which predatory water bugs (*Belostoma flumineum*) were always absent, always present, or added and removed at different points in development. I discovered that snails formed morphological defenses against water bugs. Importantly, after the initial induction of defenses, snails showed similar developmental trajectories as snails reared without predators. Further, the snails possessed wide developmental windows for inducible defenses that extended past sexual maturity. However, being induced later in development appeared to have an associated cost (i.e. decreased shell thickness) that was not found when water bugs were always present. This epiphenotype (i.e. new shell formation as an extension of the current shell) suggests that resource limitation plays an important role in responses to temporal variation in predation risk and may have critical ecological costs that limit the benefits of the inducible defense. Lastly, the ability of snails to completely reverse their defenses was limited to early in ontogeny due to the constraints associated with modular growth of shell

material. In sum, I demonstrate that taking a developmental perspective is extremely valuable for understanding the ecology of inducible defenses.

3.2 INTRODUCTION

Predator-induced plasticity has received a great deal of attention due to the astounding diversity of prey responses to their predators including changes in behavior, morphology, and life history (Kats and Dill 1998, Tollrian and Harvell 1999). While interest in predator-induced plasticity has increased, our studies have often overlooked the importance of development in the formation of inducible defenses during an individual's lifetime (West-Eberhard 2003). The link between development and phenotypic plasticity is clear; environmentally induced phenotypes require time to form and adaptive strategies can change over ontogeny (Schlichting and Pigliucci 1998, Gilbert 2001, West-Eberhard 2003). Research in non-predator prey systems has lead the way in documenting developmental plasticity within and across species (Hensley 1993, Pigliucci and Schlichting 1995, Gedroc et al. 1996, Smits et al. 1996, Winn 1996, Huber et al. 1999, Thompson 1999). Our challenge is to determine how integrating a developmental perspective can improve our understanding of the ecology and evolution of inducible defenses.

Ecologists are increasingly aware that organisms can alter suites of traits in the face of environmental variation (Boersma et al. 1998, Pigliucci and Preston 2004). Given that most traits have a developmental component, several authors have called for more integrative studies that include multiple developmental stages, multiple traits, and multiple environments (Schlichting and Pigliucci 1998, Pigliucci 2003, West-Eberhard 2003, Relyea 2004, Boege and Marquis 2005). Such integrative studies are necessary to understand prey defensive strategies. For example, as prey grow into size refuges, they may no longer employ costly behavioral or morphological defenses (e.g., energetic and maintenance costs; Havel and Dodson 1984, Brönmark and Pettersson 1994). Further, ontogeny plays an important role in how plants induce and allocate secondary chemicals after herbivory (Zangerl and Rutledge 1996, Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000). In short, a developmental approach permits a more complete understanding of prey defensive strategies.

A developmental perspective is also needed to examine how organisms respond to temporal environmental variation. During an individual's lifetime the environment may change state at any time in development (including reverting back to an earlier state). If alternative phenotypes are adaptive solutions to different environments, theory predicts that individuals that track environmental change will be favored by selection (Gabriel 1999, Gabriel et al. 2005). Therefore, when organisms encounter temporal environmental variation, we might expect wide developmental windows for trait formation (Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000) and trait reversal (Stenson 1987, Brönmark and Pettersson 1994, Piersma and Lindström 1997, Trussell 1997, Yamada et al. 1998, Kuhlmann et al. 1999, Marchinko 2003, Rohde et al. 2004) where a developmental window is defined as the length of time during ontogeny in which a phenotype can be expressed in response to a changing environment (i.e. a wide developmental window implies that a trait is inducible over most of ontogeny). However, if organisms are unable to respond to frequent environmental changes during development (i.e. narrow developmental windows) and there are large costs of displaying a sub-optimal phenotype (i.e. strong phenotypic trade-offs), then selection may operate against attempts to track environmental change. The inability to respond to such fine-grained variation can occur in a number of ways including ontogenetic contingency, developmental constraints, and unresponsive sensory systems (Newman 1992, Diggle 1994, Leips and Travis 1994, Novoplansky et al. 1994, Trussell 1997, Emerson 2000). In predator-prey systems, we know that many prey defenses are phenotypically plastic, but the developmental windows associated with the formation and reversal of these responses are relatively unexplored (Harvell 1991, Kats and Dill 1998, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Ohnmeiss and Baldwin 2000, Van Buskirk 2002, Relyea 2003). Thus, there is a clear need to examine how development affects suites of inducible defenses when individuals experience fine-grained environmental variation.

To address changing phenotypic strategies over ontogeny and the importance of developmental windows, I examined predator-induced plasticity in a system of freshwater snails (*Helisoma trivolvis*) and predatory water bugs (*Belostoma flumineum*). Water bugs are a major snail predator in fishless habitats (Kesler and Munns 1989) and previous work in this system (using the constant presence and absence of caged predators) has found that water bugs have no effect on snail habitat use (use of the water's surface) but do induce snails to develop changes in shell shape that reduce vulnerability to water bug predation (Hoverman et al. 2005, J. T.

Hoverman and R. A. Relyea, *unpublished data*). While the defenses reduce predation rates with water bugs, they come at the cost of delayed reproduction (Hoverman et al. 2005) and increased vulnerability to attack by crayfish (J. T. Hoverman and R. A. Relyea, *unpublished data*). Thus, variation in the predator environment may play an important role in favoring plasticity in snails. This system is also excellent for examining how prey respond to temporal variation in predation risk because water bug densities are generally low in May (< 0.5 adults/m²) but can increase dramatically by July (e.g., 14 adults/m²) due to reproduction and migration from permanent overwintering ponds to more ephemeral ponds (J. T. Hoverman, E. E. Werner, D. K. Skelly, K. L. Yurewicz, and R. A. Relyea, *unpublished data*). The short generation time of snails also allows an examination of developmental windows that may extend into sexual maturity. Lastly, snails provide a unique opportunity to examine the flexibility and constraints associated with phenotypic responses in a species with accretionary (i.e. modular) growth which should constrain morphological responses to temporal variation to early in ontogeny when the potential for shape change is maximal. In this experiment, I quantified developmental trajectories of snails exposed to the constant presence and absence of caged waters bugs as well as water bug colonization and emigration at different times in development. I hypothesized that: 1) the anti-predator phenotypic strategies of snails will change over ontogeny, 2) predator induction of snails will be restricted to early stages of development (i.e. narrow developmental windows), and 3) the reversal of predator-induced phenotypes will be restricted to early stages of development.

3.3 METHODS

The experiment was conducted in an open field at the University of Pittsburgh's Aquatic Research Facility in Linesville, PA. I began by collecting 120 adult snails on 28 March 2003 from Geneva Pond #1 (a permanent pond located in northwestern PA). I placed 20 adults into each of six 100-L wading pools filled with well water to oviposit. Egg deposition began in mid-April and continued until 1 May, at which time the adults were removed from the pools. Snails hatched from 15-29 May and were fed rabbit chow ad libitum until the start of the experiment. I designed a completely randomized experiment that simulated four conditions: 1) predators never present (i.e. constant no-predator), 2) predators always present (i.e. constant predator), 3)

predators colonizing at four different times, and 4) predators emigrating at four different times. Although I use the phrase “constant-predator treatment” to describe snails reared with caged predators throughout the experiment, it is important to note that these snails did not experience predators since hatching. Predator colonization and emigration occurred on days 7, 14, 21, and 28 of the experiment and the experiment was terminated on day 35. During these 5 wks, I repeatedly observed (i.e. once per week) snail phenotypes during ontogeny. This design resulted in 10 treatments that were replicated six times for a total of 60 experimental units. The experimental units were 100-L pools that were filled with well water on 13 June. I added 5 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing zooplankton, phytoplankton, and periphyton. I also added a 16x16 cm clay tile in the center of each pool to serve as structure. On 19 June, 60 hatchling snails (mean mass \pm 1 SE, 30 mg \pm 3 mg) were added to each pool when they were approximately 4 wks old. At this size (30 mg), young snails can be safely handled without crushing yet they have only grown to 10% of their adult mass.

Each pool contained a single predator cage constructed of 10 cm sewer pipe capped with fiberglass window screen on each end to permit the chemical cues from predation to diffuse throughout the pool without allowing the predators to kill the target animals. Using predator cages allows ecologists to examine the induction effect of predators separate from the thinning effects of predators (Chivers and Smith 1998, Kats and Dill 1998, Tollrian and Harvell 1999, Relyea 2002). For the treatments assigned a predator, one adult water bug was added to the cage. Each predator was fed 400 mg of snail biomass (approximately 2-3 snails) three times per week. To equalize disturbance across treatments, cages in the no-predator treatments were lifted and immediately replaced. Every 7 d, I simulated predator colonization and emigration by removing and adding predators to the appropriate pools. Although I removed the predators from the emigration treatments, there is the potential for predator kairomones to remain in the pools. However, chemical cues released by predators tend to breakdown quickly (i.e. 1-2 d) after predators are removed (Van Buskirk 2002, Relyea 2003, Turner and Montgomery 2003). After the predators were switched, all predators were fed.

I observed two behavioral responses (i.e. habitat use) every 7 d before the cages were switched between treatments. For each pool, I counted the number of snails seen, the number that were under structure (i.e. the clay tile or predator cage), and the number within 3 cm of the water’s surface. I then calculated the fraction of snails using structure and the surface as the two

response variables. After inspecting the data, less than 1% of the snails were observed at the surface across the experiment. Therefore, this variable was excluded from the analysis.

After habitat use was recorded, I removed 10 individuals from each pool and preserved them in 10% formalin for morphological analysis. I decided to use a repeated sampling method instead of destructive sampling to reduce the number of experimental units (60 versus 300 experimental units, respectively). Sampling snails over time without replacement causes an increase in per-capita resources over time, which induces shells that are relatively narrow and high but does not affect the formation of predator-induced defenses (Hoverman et al. 2005). The preserved snails were blotted dry, weighed to the nearest milligram and measured using digital imaging software (Optimas Co., Bothell, WA). I measured four linear shell dimensions: shell width and height and aperture width and height (see Fig. 1 in Hoverman et al. 2005). I also measured shell thickness at the leading edge of the aperture using digital calipers.

On day 21 of the experiment (7 wks post-hatching), the pools were monitored for egg masses. Unfortunately, 46 of the 60 pools already contained egg masses at this time. Therefore, I was unable to collect adequate data to assess time to reproduction, but I was able to count the number of egg masses deposited in the experiment. Roughly every third day I counted the number of egg masses and then removed them. The total number of egg masses oviposited in each pool was used as my response variables. Because the number of egg masses is strongly correlated with the number of eggs (Hoverman et al. 2005), counting egg masses provided an unbiased assessment of snail fecundity. After 35 d, I terminated the experiment and preserved all surviving snails. Snail survival was high across all the predator treatments (mean \pm 1 SE = 97 \pm 1%). Because the experiment was terminated before reproduction had ended, the fecundity data represent only the initial reproductive efforts of the snails in each treatment.

3.3.1 Statistical analyses

When examining morphological plasticity, one needs to account for differences related to overall size (i.e. mass). While shell thickness showed no relationship to mass, the shell and aperture dimensions were positively related to mass. To improve the linearity of the relationships, I conducted transformations that were specific to each trait. Mass was raised to the power of 0.14 to improve the relationship with shell width while mass was raised to the power of 0.25 with

aperture width. Both shell height and aperture height showed saturating relationships with mass. Thus, I used Michaelis-Menten equations to provide transformations of snail mass that improve its linearity with shell and aperture height. To account for size variation, I regressed the four linear measurements of all individuals against their transformed mass and I saved the residuals. I then calculated the mean residual for each pool within each time period. After inspecting the data, it was clear that the four size-adjusted linear traits showed similar trends. Thus, to reduce the number of variables, I conducted a principal components analysis with the four size-adjusted traits using the pool means across the five time periods ($n = 300$). The first principal component (i.e. PC-1) had an eigenvalue of 2.4 and explained 61% of the variation. The remaining PCs had eigenvalues smaller than one and were not extracted. All four traits loaded strongly positive on PC-1 (i.e. loading ≥ 0.677). Thus, the PC-1 score for each pool across the time periods was saved and used as the response variable for shell and aperture shape.

I conducted two analyses on the data. First, because pools were sampled repeatedly, I conducted a repeated-measures multivariate analysis of variance (MANOVA) using snail behavior, mass, PC-1 (i.e. shell and aperture shape), and shell thickness to test for the effects of time, predator treatment, and their interaction. Significant multivariate tests were followed by univariate tests of significance using the Huynh-Feldt degrees of freedom correction (because the sphericity assumption was violated). If a response variable was significant following the univariate test, I conducted mean comparisons using Fisher's LSD test.

I had several objectives with my comparisons. First, I wanted to determine how each trait changed over time (i.e. developmental trajectories). Second, I wished to determine if and when the no-predator and constant-predator treatments differed. The third objective was to determine whether predator colonization caused a divergence from the constant no-predator treatment and a convergence to the constant predator treatment. The final objective was to determine whether predator emigration caused a divergence from the constant predator treatment and a convergence to the constant no-predator treatment.

For the second analysis, I used an ANOVA to examine the effects of the predator treatments on the total egg production of the snails during the experiment.

3.4 RESULTS

The MANOVA on snail behavior, mass, shell shape (i.e. PC-1), and shell thickness detected significant multivariate effects of predator treatment, time, and their interaction (Table 3.1). For each trait, I present the results in light of my four objectives. First, I considered how snails used structure. Univariate tests indicated that there was no main effect of predators but there was an effect of time and a treatment-by-time interaction (Table 3.1, Fig. 3.1A-C). Within the no-predator treatment, snails spent more time under structure in wk 1 compared to the other four weeks ($P < 0.001$). Snails also spent more time under structure in wk 2 compared to wks 3-5 ($P \leq 0.002$). There were no differences in snail behavior from wks 3 to 5 ($P \geq 0.450$). Within the constant predator treatment, snails spent more time under structure in wk 1 compared to the other four weeks ($P \leq 0.035$). Snail behavior was not different in wk 2 compared to wks 3 and 4 ($P \geq 0.302$), but snails spent more time under structure in wk 2 compared to snails in wk 5 ($P = 0.013$). There was no difference in snail behavior from wks 3 to 5 ($P \geq 0.075$). In comparing the two constant treatments within each week, the predator treatment induced snails to decrease their use of structure by 15% in wk 1 ($P < 0.001$), but not over the remainder of the experiment ($P > 0.12$). In sum, predator effects on snail behavior were limited to early in ontogeny.

I next examined the effects of predator colonization on snail behavior (Fig. 3.1B, Table 3.2). After every colonization event (i.e. wks 1, 2, 3, and 4), snail behavior simply followed the trajectory of the constant no-predator treatment ($P > 0.2$). For example, snails experiencing predator colonization and no colonization in wk 1 both reduced their use of structure by wk 2 ($P = 0.001$), and the magnitudes of these reductions were similar between treatments ($P = 0.706$). In short, the four colonization treatments never diverged from the constant no-predator treatment. Moreover, the four colonization treatments were never different from the constant predator treatment. Thus, predator colonization had no effect on snail behavior.

I then examined the effects of predator emigration on snail behavior (Fig. 3.1C, Table 3.3). After nearly every emigration event, snail behavior followed the trajectory of the constant predator treatment. There was only one exception to this pattern; when snails experienced predator emigration in wk 1, snail use of structure was greater than the constant predator treatment by wk 2 ($P = 0.038$) but not during any of the subsequent weeks ($P > 0.3$). In the other emigration treatments, snail behavior never differed from the constant predator treatment ($P >$

0.05). Moreover, the four emigration treatments were never different from the constant no-predator treatment. Thus, predator emigration had minor effects on snail behavior.

Snail mass exhibited no main effect of predators but was affected by time and the treatment-by-time interaction (Table 3.1). Within each constant treatment, snail mass increased significantly each week ($P \leq 0.030$). Averaged across both constant treatments, snail mass increased 4-fold during the experiment. In comparing the two constant treatments at each week, snails reared in constant-predator and constant-no-predator treatments only differed in mass during wk 3 (when snails in the former treatment were more massive; $P = 0.004$; Fig. 3.1D). Thus, predators did not have strong effects on snail mass.

I next examined the effects of predator colonization on mass (Fig. 3.1E, Table 3.2). After 1 wk of living without predators, snails experiencing either predator colonization or no colonization both exhibited increased mass the following week ($P < 0.001$), but the magnitude of the increase was similar between treatments ($P = 0.134$). In subsequent weeks, these colonized snails had greater mass than the constant no-predator treatment during wks 3-4 ($P < 0.04$) but this difference eroded by wk 5 ($P = 0.469$). After 2-4 wks of living without predators, snails experiencing predator colonization exhibited a mass that was always similar to the mass of snails in the constant no-predator treatment ($P > 0.06$). Compared to the constant predator treatment, snail mass eventually converged on the constant predator treatment if colonization occurred during wks 1-3 ($P > 0.09$) but not if colonization occurred during wk 4 ($P = 0.003$). Thus, predator colonization had limited (and often ephemeral) effects on snail mass.

I then examined the effects of predator emigration on mass (Fig. 3.1F, Table 3.3). For all four emigration treatments, snail mass always followed the same trajectory as snails that experienced a constant predator environment ($P > 0.19$). Moreover, the increase in snail mass over time in the four emigration treatments was never different from the constant no-predator treatment ($P > 0.07$). Thus, predator emigration had no effect on snail mass.

When I conducted a PCA on snail morphology, I found that higher scores on PC-1 were associated with relatively higher and wider shells and apertures (for simplicity, I will refer to higher PC-1 scores as relatively larger shells). Snail shape (i.e. PC-1) was affected by predators, time, and the treatment-by-time interaction (Table 3.1). Within the constant no-predator treatment, shell size increased from wk 1 to wk 2 ($P = 0.001$). By wk 3, shells became relatively smaller compared to wk 2 ($P = 0.002$) and similar to wk 1 ($P = 0.593$). In wks 4 and 5, shells

were smaller than wks 1-3 ($P < 0.001$) but not different from each other ($P = 0.984$). Within the constant predator treatment, relative shell size increased from wk 1 to wk 2 ($P = 0.001$). At wk 3, shells were similar to wk 2 ($P = 0.410$) but larger than wk 1 ($P = 0.001$). In wks 4 and 5, shells were relatively smaller than wks 2-3 ($P < 0.001$), similar to wk 1 ($P \geq 0.197$), and not different from each other ($P = 0.269$). Comparing the two constant treatments within each time period (Fig. 3.2A), snails were similar in shell size at wk 1 ($P = 0.354$), but the constant predator treatment induced larger shells during wks 2-5 ($P \leq 0.048$). An additional repeated-measured ANOVA using only the data from wks 2-5 confirmed the effects of predators ($F_{1,10} = 15.9$, $P = 0.003$) and time ($F_{3,8} = 36.8$, $P < 0.001$) but no interaction ($F_{3,8} = 1.3$, $P = 0.331$). Hence, after the initial induction of relatively larger shells, the developmental trajectories of the two treatments were similar.

I next examined the effects of predator colonization on shell size (Fig. 3.2B, Table 3.2). After living without predators for 1 wk, snails experiencing either predator colonization or no colonization both exhibited increased shell size the following week ($P = 0.001$), but the magnitude of the increase was greater in the colonization treatment ($P = 0.002$). In subsequent weeks, this colonization treatment followed the trajectory of snails in the constant predator treatment ($P > 0.3$) and remained different from the constant no-predator treatment ($P \leq 0.005$). After living without predators for 2 wks, shell size remained similar the following week ($P = 0.176$) in the colonization treatment but decreased in the constant no-predator treatment ($P = 0.055$), producing a difference in shell shape between these two treatments in wk 3 ($P = 0.015$). The subsequent trajectory followed the trajectory of snails experiencing constant predators ($P > 0.08$) and remained different from the constant no-predator treatment ($P \leq 0.002$). After living without predators for 3 wks, shell size declined the following week in both the colonization and constant no-predator treatments ($P \leq 0.008$) and the two treatments did not differ ($P = 0.183$) at wk 4. By wk 5, however, snails in this colonization treatment diverged from the constant no-predator treatment ($P = 0.002$) and converged onto the constant predator trajectory ($P = 0.232$). After living without predators for 4 wks, shell size did not change in either the colonization or constant no-predator treatments by wk 5 ($P > 0.6$). However, this colonization treatment was not different from the constant predator treatment ($P = 0.108$). In summary, predator colonization in wks 1-3 lead to the formation of relatively larger shells that diverged from the constant no-predator trajectory and converged upon the constant predator trajectory.

I then examined the effects of predator emigration on shell size (Fig. 3.2C, Table 3.3). After living with predators for 1 wk, snails experiencing emigration or constant predators both increased their shell size the following week ($P \leq 0.005$). However, the increase in shell size was smaller in the emigration treatment than in the constant predator treatment ($P = 0.012$) and converged upon the constant no-predator treatment ($P = 0.157$). Throughout the subsequent weeks, snails in the wk-1 emigration treatment followed the same trajectory as snails in the constant no-predator treatment ($P > 0.5$) and were always smaller than snails in the constant predator treatment ($P < 0.03$). After living with predators for 2 wks, snails experiencing predator emigration exhibited a decline in shell size the following week ($P = 0.002$), snails experiencing constant predators exhibited no change ($P = 0.410$) and the two treatments were different from each other by wk 3 ($P = 0.002$). In the subsequent weeks, snails in the wk-2 emigration treatment were always similar to the constant no-predator treatment ($P > 0.25$) and remained different from the constant predator treatment at wk 4 ($P = 0.005$) but not at wk 5 ($P = 0.385$). After living with predators for 3 wks, snails experiencing predator emigration or constant predators both exhibited a decline in shell size the following week ($P \leq 0.001$) and the declines followed a similar trajectory through wks 4 and 5 and were of similar magnitude ($P > 0.5$). The wk-3 emigration treatment was different from the constant no-predator treatment on wk 4 ($P = 0.001$) but not on wk 5 ($P = 0.148$). After living with predators for 4 wks, snails experiencing predator emigration did not change shell size the following week ($P = 0.130$) and did not differ in shell size from either of the two constant treatments ($P > 0.1$).

Univariate tests indicated that predators, time, and the treatment-by-time interaction affected shell thickness (Table 3.1). Within each constant treatment, shell thickness increased significantly each week ($P \leq 0.057$). Averaged across both constant treatments, thickness increased by 23-fold. In comparing the two constant treatments within each week (Fig. 3.2D), snails reared with predators were slightly thinner than snails reared without predators in wk 2 ($P = 0.011$) but not during the other weeks ($P \geq 0.181$). Thus, the constant predator environment had minor effects on shell thickness.

I then examined the effects of predator colonization on shell thickness (Fig. 3.2E, Table 3.2). After living without predators for 1 wk, snails experiencing no predators or predator colonization both exhibited increased shell thickness the following week ($P < 0.03$), but the increase was smaller in the colonization treatment ($P = 0.01$). In subsequent weeks, this

colonization treatment was never different from the trajectories of the two constant treatments ($P > 0.2$). After living without predators for 2 wks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.001$) but not if the snails experienced predator colonization ($P = 0.122$). In subsequent weeks, this colonization treatment always had thinner shells than the constant no-predator treatment ($P < 0.04$) and generally had thinner shells than the constant predator treatment (wk 4, $P = 0.001$; wk 5, $P = 0.084$). After living without predators for 3 wks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.001$) but decreased if the snails experienced predator colonization ($P = 0.004$). The colonization treatment was different from the constant no-predator treatment ($P = 0.001$). In the subsequent week, this colonization treatment had thinner shells than either constant treatment ($P = 0.001$). After living without predators for 4 wks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.021$) but decreased if the snails experienced predator colonization ($P = 0.019$). The colonization treatment induced thinner shells than both of the constant treatments ($P \leq 0.003$). In summary, when predators colonized midway to late in ontogeny, snails developed much thinner shells.

Next, I examined the effects of predator emigration on shell thickness (Fig. 3.2F, Table 3.3). After living with predators for 1 wk, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P < 0.001$). The increase in shell thickness was greater in the emigration treatment than in the constant predator treatment during wk 2 ($P = 0.009$) but not during the subsequent weeks ($P > 0.09$). Shell thickness remained similar between this emigration treatment and the constant no-predator treatment throughout the experiment ($P > 0.09$). After living with predators for 2 wks, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P < 0.001$), but the emigration treatment induced a slightly thicker shell ($P = 0.050$). In the subsequent weeks, this emigration treatment was not different from either of the two constant treatments ($P \geq 0.07$). After living with predators for 3 wks, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P \leq 0.003$) and the increase was similar in magnitude ($P = 0.507$). The emigration treatment was not different from the constant no-predator treatment ($P = 0.663$). In the subsequent week, this emigration treatment was not different from either of the two constant treatments ($P > 0.7$). After living with predators for 4 wks, shell thickness increased the following week if snails experienced constant predators or

predator emigration ($P \leq 0.047$) and the increase was similar in magnitude ($P = 0.888$). The emigration treatment was not different from the constant no-predator treatment ($P = 0.789$). In summary, predator emigration always induced a thickness that was similar to or greater than the two constant treatments.

The last analysis examined the total egg production of snails. Egg production was not significantly affected by the predator treatments ($F_{9,50} = 1.2$, $P = 0.293$, Fig. 3.3). However, there was a trend in which the constant predator treatment produced fewer egg masses than the constant no-predator treatment ($P = 0.072$).

3.5 DISCUSSION

I discovered that snails are amazingly flexible in the formation of anti-predator defenses over ontogeny. By observing phenotypic responses across development, one can obtain an understanding of how prey integrate suites of defensive traits (Brönmark and Pettersson 1994, Arnqvist and Johansson 1998, Relyea 2003). In this study, water bugs induced a short-lived behavioral response in snails. During the first week of the experiment, water bugs induced a 15% decrease in the use of structure by snails but this response did not persist. The use of structure by snails is known to be predator-specific. For example, predatory crayfish spend much of their time under structure and induce snails to move away from structure whereas fish spend much of their time in the water column and induce snails to move into structure (Turner et al. 1999). In contrast, water bugs in natural ponds feed throughout the water column (i.e. at the surface and under refuges). Therefore, it is unlikely that the avoidance of structure is an adaptive strategy that enables snails to escape predation by water bugs.

Snails also expressed morphological changes with water bugs that included larger shells and larger apertures. Although predators affected the overall developmental trajectory of shell size, snails that were induced by predators were subsequently followed a similar developmental trajectory as the uninduced snails. This suggests that snail defenses are induced early in development and the relative magnitude of the defense is maintained over time. The formation of larger shells is generally consistent with laboratory and mesocosm experiments that examined how the constant presence of water bugs affected snail final morphology (Hoverman et al. 2005,

Hoverman and Relyea, *in review*). I have recently tested the adaptive value of water bug-induced traits and found several clear trends: 1) water bugs impose strong selection for relatively larger shells, 2) snails with relatively larger shells escape predation over a wide range of body sizes, and 3) large body size alone is not an effective strategy for escaping predation from water bugs (J. T. Hoverman and R. A. Relyea, *unpublished manuscript*). Thus, for snails to escape predation by water bugs, they must form and maintain a relatively larger shell as they develop. In addition to morphology, I also found that snails were 21% larger in wk 3 when reared with water bugs. Previous work has shown that snails will delay reproduction and reach a larger size at reproduction in the presence of water bugs (Hoverman et al. 2005). However, once reproduction begins, snails induced by water bugs exhibit a higher rate of egg production that quickly converges on the egg production of the no-predator phenotype (Hoverman et al. 2005). While I did not find significant differences in egg production, there was a trend for snails exposed to water bugs to lay fewer egg masses, suggesting that reproduction was somewhat delayed in the predator treatment.

Recently, ecologists have become more aware that phenotypic plasticity can occur in multiple traits and follow different developmental trajectories. For example, some prey use behavioral defenses early in ontogeny that allow immediate avoidance of predators and provide the time necessary to form morphological defenses. After morphological defenses are formed, prey can rely less on costly behavioral defenses (e.g., reduced growth rates, DeWitt et al. 1999, Rundle and Brönmark 2001, Relyea 2003, Cotton et al. 2004). Such results underscore the importance of quantifying developmental trajectories because we can identify the changing strategies of organisms (Pigliucci and Schlichting 1995, Boersma et al. 1998, Baldwin 1999, Donohue and Schmitt 1999, Sultan 2000). Indeed, because there can be genetic variation for developmental trajectories, we need to consider the potential for natural selection to operate on the shape of the trajectory rather than simply the phenotype at a single point in time (Gedroc et al. 1996, Pigliucci et al. 1997, Cheplick 2003). In sum, the explicit inclusion of development into plasticity research will be imperative to understanding how organisms integrate their phenotypic responses in the face of environmental variation.

Numerous studies have investigated predator-induced plasticity by exposing prey to the constant presence or absence of predators, an approach that adequately represents spatial variation in predation risk but overlooks the importance of temporal variation in predation risk.

For many organisms, the state of the environment can change during development (i.e. within-generation or fine-grained variation). If possessing the wrong phenotype in a given environment is costly, then selection should favor individuals with wide developmental windows that allow induction throughout ontogeny (Schlichting and Pigliucci 1998, Gabriel 1999, Gabriel et al. 2005). In this system, water bugs colonize semi-permanent ponds in mid-May to early-June. Given that snails can develop into a reproductive adult within 2 to 3 months, they can experience water bug colonization at any time from hatching through adulthood. I found that as long as water bugs colonized before the fourth week of the experiment, snails could produce larger shells. Interestingly, the speed of trait induction was more rapid when water bugs colonized early. For example, snails experiencing water bug colonization in wks 1 and 2 formed shells that were as large as the constant-predator treatment within one week. In contrast, snails experiencing water bug colonization in wk 3 required 2 wks to converge onto the constant-predator phenotype. Importantly, the lack of significant growth during the week following water bug colonization may underlie the delayed induction of relatively larger shells. Such a slow rate of defensive trait induction may lead to higher rates of predation until the defense is formed. More work is needed in this system to determine how effective these defenses are at reducing predation rates at different points in trait development. Overall, snails possess relatively wide developmental windows for their inducible defenses, suggesting that there are substantial costs of possessing a no-predator phenotype in an environment containing predatory water bugs (i.e. a high probability of mortality).

While developmental windows for induced defenses have frequently been examined in plants (Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000), the few existing studies in animal systems have found that developmental windows can be either narrow (Harvell 1991) or wide (Kats and Dill 1998, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Van Buskirk 2002, Relyea 2003). Likewise, in non-predatory systems, the size of developmental windows varies for both plant and animal systems (Newman 1992, Diggle 1994, Leips and Travis 1994, Novoplansky et al. 1994, Trussell 1997, Emerson 2000, Weinig and Delph 2001, Sachs 2002). Evolutionarily, narrow developmental windows pose possible limitations to the benefits of phenotypic plasticity because they decrease the accuracy of matching the environment and this may result in selection against phenotypic plasticity (Moran 1992, Padilla and Adolf 1996, DeWitt et al. 1998, Tollrian and Harvell 1999, Gabriel et al. 2005). The incorporation of natural

patterns of temporal environmental variation into our experiments will continue to provide valuable insights into the ecology and evolution of phenotypic plasticity.

The inclusion of temporal variation in predation risk also allowed me to document a unique effect on shell thickness that would have been masked by simply examining phenotypes in constant predator and no-predator treatments. When water bugs colonized after wk 1, snails developed 7-77% thinner shells (Fig. 3.2E). Interestingly, snails were not constrained to have extremely thin shells throughout the remainder of development (Fig. 3.4). When water bugs colonized in wk 2, snails produced a large shell by wk 3 (i.e. similar to the constant-predator treatment) and in wks 4 and 5 their shell thickness increased. Another important result occurred when water bugs colonized in wk 4. While I did not observe significant induction of larger shells in wk 5, snails did produce shells that were thinner. This suggests that given more time (i.e. an additional week) snails may have formed larger shells since the shells were becoming thinner. Overall, these results demonstrate that complete induction in one trait at different points in ontogeny does not imply complete induction of all traits. Moreover, these findings may be explained by resource limitation that frequently affects induced defenses in plants and animals (Karban and Baldwin 1997, Tollrian and Harvell 1999). Snails directly absorb the majority of the calcium needed for shell formation from their environment and produce thicker, larger shells with higher calcium availability (Russell-Hunter 1978, Brodersen and Madsen 2003). Given that my mesocosms are closed systems, calcium availability was likely more reduced late in the experiment, preventing snails from simultaneously forming a thicker shell and a larger shell when water bugs colonized. While further work is needed in this system, resource limitation may play a critical role in the ability for organisms to respond to temporal environmental variation.

When selection favors wide developmental windows, there can be fitness costs associated with phenotypes induced later in ontogeny (DeWitt et al. 1998, West-Eberhard 2003). The production of larger, thinner shells when water bugs colonized late in ontogeny may represent an epiphenotype (i.e. new shell formation as an extension of the current shell; DeWitt et al. 1998). Such add-on phenotypes can have two possible consequences for prey. First, the epiphenotype may be less effective at defense against water bugs than phenotypes induced early in ontogeny. In this case, the epiphenotype would have lower fitness compared to phenotypes produced in constant environments. Second, the epiphenotype may make prey more vulnerable to other

predators. For example, background responses to one predator may render prey easier to handle and consume by a second predator or place prey on developmental trajectories that constrain future responses to predators. Thus, prey may find themselves in a precarious situation in which they must balance their responses to predators that kill by using different strategies. In this system, crayfish attack snails by chipping the aperture or crushing the shell. Thus, snails with thinner shells as a result of water bug colonization may be extremely vulnerable to attack by crayfish. While possessing wide developmental windows allows flexibility in phenotypic responses, organisms may incur additional costs that are not associated with phenotypes produced in constant environments

The removal of inducing cues can provide insights into the size of developmental windows for trait reversibility. Theory predicts that the evolution of reversibility is favored when individuals experience multiple environmental states within a generation, when the fitness cost of not matching the phenotype to the environment is large (i.e. strong phenotypic trade-offs), and when the response lag time is shorter than the duration of the environmental state (Gabriel 1999). I found relatively narrow development windows for the complete reversal of larger shells, which is in contrast to the wide developmental windows found for defensive trait formation. While a complete reversal of shell size occurred when water bugs were removed in wk 1, an incomplete reversal occurred when water bugs were removed in wks 2-4.

The lack of reversibility later in ontogeny could be explained in several ways. First, snails may be reluctant to reverse defenses if they are uncertain that the predator has left the habitat (Sih 1992, Van Buskirk 2002). Delaying the reversal of defensive phenotypes would be an adaptive strategy that reduces the probability of attack from an undetected predator (Van Buskirk 2002). Alternatively, snails may experience developmental constraints associated with shell formation because the shape of previously deposited shell cannot be altered (Stone 1995). For example, when I examined the raw data (i.e. before size-correction) from the study, final shell width in the no-predator treatment was 1.13 ± 0.02 cm. When water bugs emigrated late in the experiment, shell width was already > 1.2 cm, prohibiting any reversal to the no-predator shell width. In contrast, snail shells were quite small early in development (wk 1 shell width = 0.7 cm) allowing snails experiencing predator emigration to reverse to the no-predator phenotype. Developmental constraints for trait reversal may also be linked to how the organism produces the trait. For example, tadpoles are able to reverse their morphological defenses (i.e.

smaller bodies and deeper tails) because they simply shunt more resources to the growth of the body and thereby reduce the relative size of their tail (Van Buskirk 2002, Relyea 2003). Modular traits such as plant stems or gastropod shells may be more developmentally constrained because the shape of previously formed structures cannot be altered. Thus, there may be limits on the reversibility of inducible defenses resulting from how the defenses were developed. Overall, the reversibility of defensive traits will depend upon the past history of phenotypic responses, the assessment of predation risk, the fitness benefit of reversing, and the mode of trait production (Sih 1992, Diggle 1994, Weinig and Delph 2001, Sachs 2002, Kurashige and Agrawal 2005).

3.5.1 Conclusions

Evolutionary biologists are aware of the developmental component of inducible defenses, yet few studies have rigorously incorporated a developmental perspective. Such studies are imperative for understanding developmental trajectories of different traits, allocation trade-offs, developmental windows, and phenotypic reversibility. By taking a developmental approach, I discovered that freshwater snails possess wide developmental windows for predator induction but narrow windows for reversibility due to the constraints of modular growth. Moreover, incorporating temporal variation in the predator environments illuminated important responses that could not be observed in traditional experiments incorporating only spatial variation in predator environments. Through the incorporation of both spatial and temporal heterogeneity, we can obtain a more complete understanding of how organisms have evolved to make their phenotypic decisions and how these decisions affect the ecology of the system.

Table 3.1 Results of repeated measures MANOVA on the effects of predator colonization and emigration on the behavior, mass, shell and aperture shape (PC-1) and shell thickness of snails over time (A). Univariate tests (*P*-values) of time and predator*time are conducted using the Huynh-Feldt degrees of freedom correction factor because the assumption of sphericity was violated (B).

A. Multivariate tests	df	F	P
Predator	36, 178	4.9	<0.0001
Time	16,35	274.7	<0.0001
Predator * Time	144, 292	2.4	<0.0001

B. Univariate tests	Predator	Time	Predator * Time
Use of structure	0.259	<0.0001	0.003
Mass	0.231	<0.0001	0.028
PC-1	<0.0001	<0.0001	<0.0001
Shell thickness	<0.0001	<0.0001	<0.0001

Table 3.2 Results (*P*-values) from analysis of the effects of predator colonization at four times on snail traits. For each colonization treatment, snail phenotypes were compared to the phenotypes expressed in both constant treatments within each week after colonization.

Trait	Time of colonization	Constant treatment comparison	Time of comparison			
			Wk 2	Wk 3	Wk 4	Wk 5
Use of structure	Wk 1	No predator	0.932	0.553	0.970	0.716
		Predator	0.352	0.955	0.424	0.844
	Wk 2	No predator		0.299	0.547	0.757
		Predator		0.094	0.873	0.801
	Wk 3	No predator			0.591	0.836
		Predator			0.196	0.724
	Wk 4	No predator				0.395
		Predator				0.770
Mass	Wk 1	No predator	0.134	0.025	0.039	0.469
		Predator	0.352	0.907	0.318	0.448
	Wk 2	No predator		0.098	0.065	0.142
		Predator		0.611	0.442	0.998
	Wk 3	No predator			0.214	0.822
		Predator			0.022	0.092
	Wk 4	No predator				0.106
		Predator				0.003
PC-1	Wk 1	No predator	0.002	0.001	0.001	0.005
		Predator	0.393	0.995	0.991	0.385
	Wk 2	No predator		0.015	0.001	0.002
		Predator		0.305	0.087	0.207
	Wk 3	No predator			0.183	0.002
		Predator			0.009	0.232
	Wk 4	No predator				0.696
		Predator				0.108
Shell thickness	Wk 1	No predator	0.010	0.737	0.750	0.283
		Predator	0.310	0.774	0.435	0.503
	Wk 2	No predator		0.001	0.001	0.035
		Predator		0.001	0.001	0.084
	Wk 3	No predator			0.001	0.001
		Predator			0.001	0.001
	Wk 4	No predator				0.001
		Predator				0.003

Table 3.3 Results (*P*-values) from analysis of the effects of predator emigration at four times on snail traits. For each emigration treatment, snail phenotypes were compared to the phenotypes expressed in both constant treatments within each week after emigration.

Trait	Time of emigration	Constant treatment comparison	Time of comparison			
			Wk 2	Wk 3	Wk 4	Wk 5
Use of structure	Wk 1	No predator	0.275	0.436	0.815	0.844
		Predator	0.038	0.896	0.321	0.716
	Wk 2	No predator		0.992	0.888	0.457
		Predator		0.510	0.367	0.195
	Wk 3	No predator			0.226	0.506
		Predator			0.052	0.916
	Wk 4	No predator				0.868
		Predator				0.468
Mass	Wk 1	No predator	0.428	0.195	0.893	0.859
		Predator	0.828	0.381	0.335	0.195
	Wk 2	No predator		0.079	0.307	0.137
		Predator		0.685	0.940	0.985
	Wk 3	No predator			0.071	0.127
		Predator			0.466	0.954
	Wk 4	No predator				0.254
		Predator				0.735
PC-1	Wk 1	No predator	0.157	0.556	0.797	0.746
		Predator	0.012	0.005	0.001	0.023
	Wk 2	No predator		0.734	0.262	0.255
		Predator		0.002	0.005	0.385
	Wk 3	No predator			0.001	0.148
		Predator			0.928	0.579
	Wk 4	No predator				0.134
		Predator				0.616
Shell thickness	Wk 1	No predator	0.290	0.097	0.668	0.892
		Predator	0.009	0.088	0.130	0.785
	Wk 2	No predator		0.056	0.462	0.580
		Predator		0.050	0.070	0.338
	Wk 3	No predator			0.663	0.932
		Predator			0.507	0.747
	Wk 4	No predator				0.789
		Predator				0.888

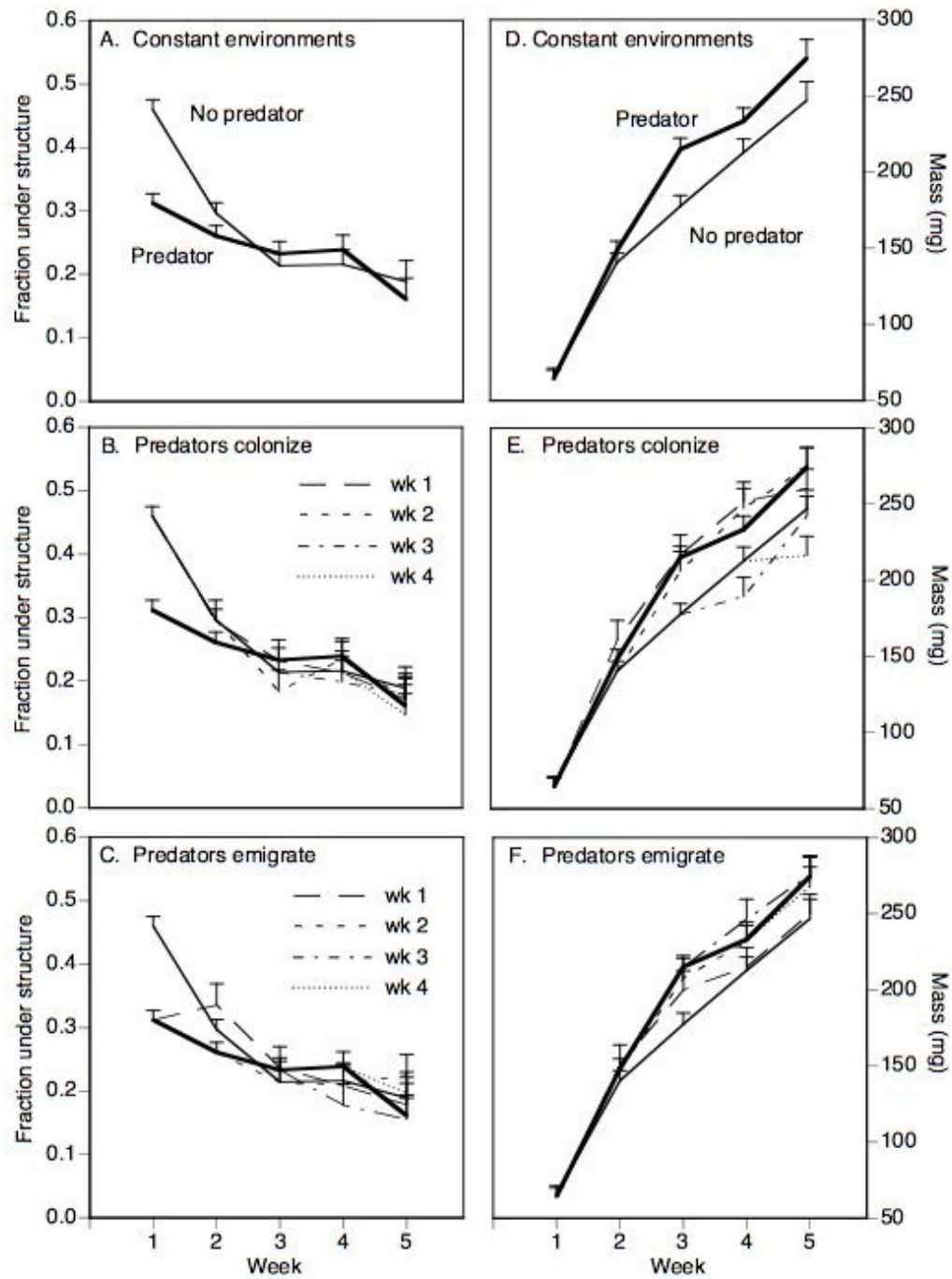


Figure 3.1 The effects of different predator treatments on snail behavior (use of structure, A-C) and mass (D-F) over time. The treatments simulated the constant presence or absence of water bugs (A, D), water bug colonization at different times (B, E), or water bug emigration at different times (C, F). Water bug colonization and emigration occurred at four different times and are represented by dashed lines. The constant presence or absence of water bugs is shown in each panel for comparison. Data are least-squares means ± 1 SE.

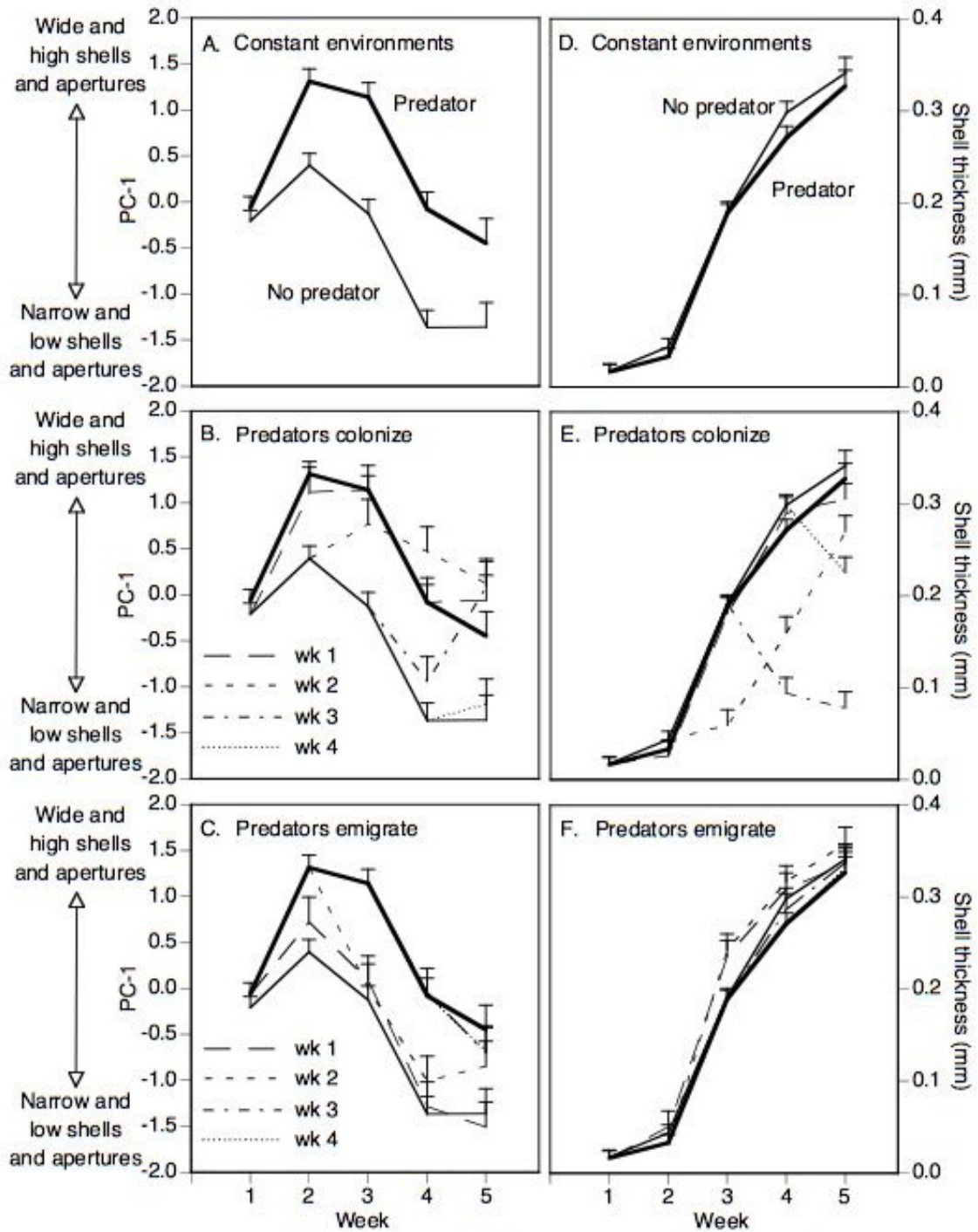


Figure 3.2 The effects of different predator treatments on PC-1 (i.e. shell and aperture shape, A-C) and shell thickness (D-F) over time. See Fig. 4.1 for a description of the treatments. Data are least-squares means ± 1 SE.

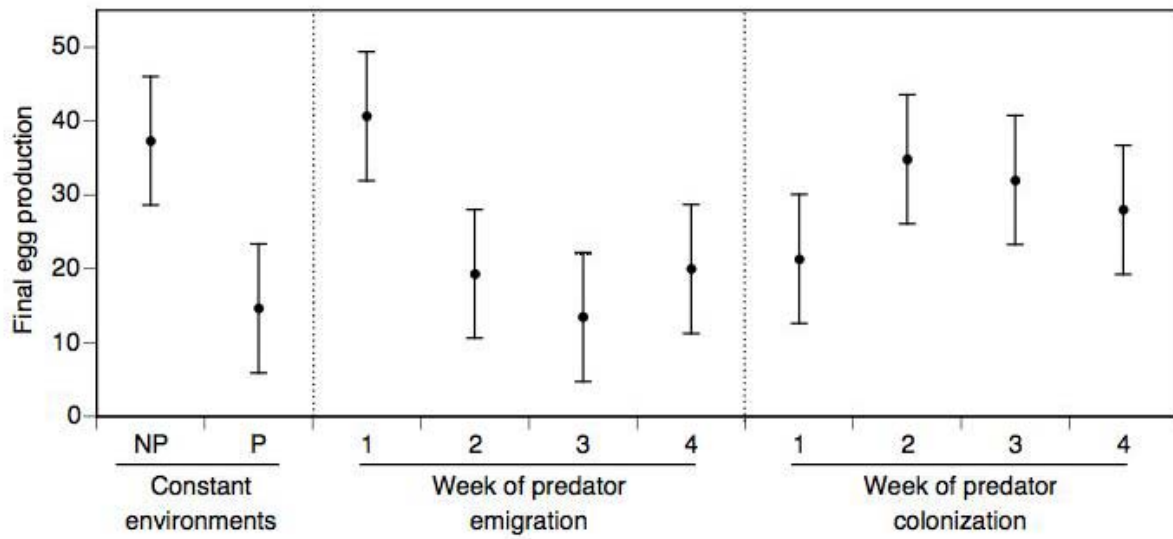


Figure 3.3 The effects of different predator treatments on the total egg production of snails. The constant no-predator (NP) and predator treatment (P) are shown in the first panel. The predator emigration and colonization treatment are displayed in the next two panels, respectively, according to the week the treatment was applied. Data are least-squares means \pm 1 SE.

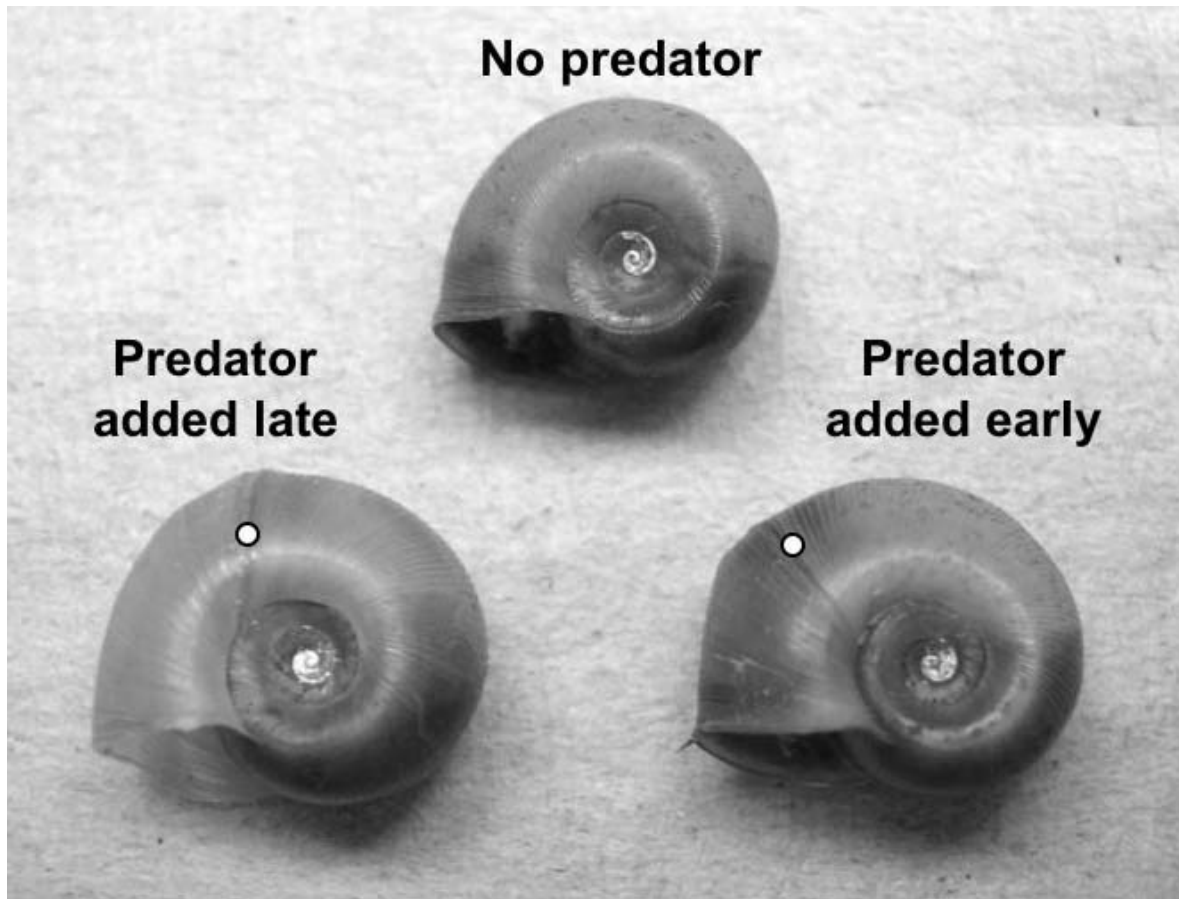


Figure 3.4 Morphological responses of *Helisoma trivolvis* to water bug colonization. The top snail was reared in the absence of water bugs while the bottom two snails experienced water bug colonization early (bottom right) and late (bottom left) in the experiment. The dots illustrate the approximate point in shell formation where the water bug was added. Note the difference in shell thickness (dark versus light gray regions) between the snails exposed to water bug colonization early compared to late in the experiment.

4.0 THE RULES OF ENGAGEMENT: HOW TO DEFEND AGAINST COMBINATIONS OF PREDATORS

4.1 ABSTRACT

Studies of inducible defenses have traditionally examined prey responses to one predator at a time. However, prey in nature encounter combinations of predators that should force them to produce phenotypic compromises. I examined how snails (*Helisoma trivolvis*) alter their phenotype with three different predator species that were present alone and in pairwise combinations. When snails were exposed to each predator alone, they formed predator-specific defenses that reflected the differences in each predator's foraging mode. When snails were exposed to pairwise combinations of predators, their phenotype was dependent on their ability to detect each predator, the risk posed by each predator, and the effectiveness of a given defense against each predator. Consequently, responses to combined predators were typically biased towards one of the predators in the pair. This suggests that prey facing combined predators do not form simple intermediate defenses and, as a result, may experience enhanced mortality risk when they encounter natural predator regimes.

4.2 INTRODUCTION

Prey rarely encounter a single predator in natural communities but coexist with a diversity of predator species that often differ functionally (Sih et al. 1998, Chalcraft and Reserits 2003, Griffen 2006). In recent years, ecologists have attempted to predict prey mortality rates with combined predators based on mortality rates with single predators. The general failure of these studies to predict prey survival with combined predators is often attributed to an incomplete

understanding of inducible defenses (Sih et al. 1998, Relyea 2003). Predator species typically differ in their foraging location or capture technique, which can lead to opposing selective pressures on prey phenotypes. Consequently, many prey species have evolved the ability to form predator-specific defenses that are integrated across morphology, behavior, and life history to cope with each predator species (Krupa and Sih 1998, Kats and Dill 1998, McIntosh and Peckarsky 1999, Tollrian and Harvell 1999, Relyea 2001). While research on inducible defenses has grown steadily over the years, research on induced defenses with combinations of predators has not kept pace. By addressing the effects of combined predators on prey phenotypes, community ecologists can obtain insights into the effects of predation on prey populations within natural communities as well as their indirect effects on other species in the community.

Ecologists have clearly established that the inducible defenses documented in a diversity of groups can be adaptive strategies for reducing the risk of predation with a given predator (Karban and Baldwin 1997, Tollrian and Harvell 1999). However, prey encounter a more complex situation when two or more predator species are present simultaneously. Not only must prey be able to detect the presence of each predator, an optimal response requires the integration of their defensive traits based on the risk posed by each predator and based on the relative effectiveness of each defense against each predator. Over the last decade, several studies have addressed prey responses to combined predators (Lima 1992; Matsuda et al. 1993, 1994, 1996, Peckarsky and McIntosh 1998, McIntosh and Peckarsky 1999, Turner et al. 2000, Relyea 2003, Wiackowski et al. 2003, Teplitsky et al. 2004). When predator species induce responses in the same direction but with different magnitudes, prey respond to combined predators by simply responding to the most risky predator in the combination (reviewed in Relyea 2003). This decision rule appears to work well because a defense against the most risky predator automatically defends the prey against less risky predators (Relyea 2003). When individual predator species induce prey defenses in opposite directions, prey generally produce intermediate phenotypes in response to multiple predators (McIntosh and Peckarsky 1999). This compromise decision with combined predators is assumed to balance the overall risk of predation. Thus, while combined predators may present a more complex situation for prey compared to encounters with a single predator, we do have clear expectations of how prey should respond to combined predators.

When prey are exposed to combined predators, they experience both a change in predator composition and a higher total predator density compared to treatments with a single predator. Traditionally, researchers have used additive experimental designs that compare the phenotypes induced by each predator alone to the phenotypes induced by both predators added together (Peckarsky and McIntosh 1998, McIntosh and Peckarsky 1999, Turner et al. 2000, Wiackowski et al. 2003, Teplitsky et al. 2005). For example, if two predators at a low density induce a trait to change in opposing directions and the combination of the two predators results in an intermediate phenotype, researchers have correctly concluded that the response was due to the predator combination and not to predator density. However, if two predators at a low density induce a trait to change in the same direction and the combination of the two predators results in a more extreme phenotype than either predator alone, it is unclear whether the more extreme response occurred due to the change in predator composition or due to the change in predator density. Unfortunately, without prior knowledge of prey responses to increases in each predator's density, predictions about responses to combined predators become difficult. Recently, it has been suggested that an additive-plus-substitutive design is a solution to this problem (Relyea 2003) because prey responses to combined predators can be compared to both single and double densities of each predator alone. While an additive-plus-substitutive design requires a larger experiment, its use will be pivotal in studies where *a priori* knowledge about prey responses is unavailable.

Freshwater snails and their predators provide an excellent study system to address these issues of combined predators. In this study, I utilized the snail *Helisoma trivolvis* and three of its most common predators: 1) water bugs (*Belostoma flumineum*), 2) crayfish (*Orconectes rusticus*), and 3) pumpkinseed sunfish (*Lepomis gibbosus*). Crayfish and sunfish are generally large predators that exist at low densities (≤ 2 adults/m², J.T. Hoverman, *unpublished data*), whereas water bugs are small predators that can reach high densities (40 adults/m²; Kesler and Munns 1989). In addition to differences in population size, each predator uses a different tactic to consume snails. Fish use their pharyngeal jaw muscles to crush shells. Water bugs invade shells using a modified mouthpart that pierces the snail's soft body. Crayfish use a variety of tactics to consume snails including shell entry, aperture chipping, and shell crushing. For this snail species, the most common tactic is aperture chipping. To counter the different feeding tactics of their predators, snails display a variety of predator-specific responses including

changes in shell shape, behavior, and life history (Snyder 1967, Crowl and Covich 1990, DeWitt and Langerhans 2003, Hoverman et al. 2005). While this system is well-suited for studying the inductive effects of combined predators, work has focused on a single snail (i.e. *Physa*), a single behavioral trait, and a single predator combination (Turner et al. 2000). I expand on this work by examining snail responses to several predator combinations as well as multiple traits (i.e. behavior, morphology, and growth).

To examine the responses of prey to predator combinations, I conducted two experiments. The first experiment examined the predation risk (i.e. mortality rate) associated with each of the three predator species. Since fish and crayfish are relatively large predators, I expected snail mortality rates to be much higher with these two predators compared to water bugs. The information obtained from this experiment was used to design the second experiment that addressed the phenotypic responses of snails to separate and combined predators. Armed with information about the interactions between snails and their predators when encountered in isolation, I can make several predictions about how snails should respond to separate and combined predators. First, snails will form predator-specific defenses that reflect the differences in predator foraging locations and feeding tactics. Second, higher densities of each predator (i.e. higher risk) should lead to more extreme defenses. Third, given that each predator induces a unique suite of traits, I expect pairwise combinations of predators to induce intermediate phenotypes that potentially balance the risk of predation from both predators.

4.3 METHODS

In the first experiment, I conducted predation trials to quantify the risk of predation associated with the three predator species (water bugs, crayfish, and sunfish) for snails of two different sizes. The experiment was conducted in nine 800-L cattle tanks containing 700 L of well water at the University of Pittsburgh's Aquatic Research Facility in Linesville, PA. Each tank contained two 30 x30 cm corrugated pipes capped with fiberglass window screen on each end. The experiment was a completely randomized split-plot design with predator species as the whole-plot factor and snail size as the split-plot factor. Snail size was broken into small (mean mass ($n = 20$) ± 1 SE = $39.1 \text{ mg} \pm 2.1 \text{ mg}$) and large ($68.1 \text{ mg} \pm 2.9 \text{ mg}$) size classes. The snails

in each size class were reared without predators (i.e. predator-naïve) from eggs in outdoor wading pools until the start of the predation trials. Within each tank for each predator species, I placed 10 snails of the appropriate size class and one predator in the cages. Each whole-plot factor was replicated three times. The main interest was the mortality rate (i.e. number consumed / hr) of snails with each predator species. Based on previous observations, crayfish and sunfish consume snails rapidly (i.e. several snails / hr). To accurately assess mortality rates, I stopped the crayfish and fish treatments after 1 hr to ensure that a few snails remained in the cages. Because water bugs require several hours to consume a single snail, water bugs were allowed to feed for 24 hrs and the mortality rate was calculated as the number of snails consumed divided by 24 hrs. Mortality rate was log transformed prior to conducting an analysis of variance (ANOVA) to satisfy the assumption of homogeneous errors.

In the second experiment, I examined the effects of combined predators on snail phenotypes using pond mesocosms. On 27 March, I collected 350 adult snails from a nearby pond and placed 25 adults into each of 14 wading pools filled with 100 L of well water to oviposit. Egg deposition began in April and continued into early May, at which time the adults were removed from the pools. Snails began hatching on 9 May and were fed rabbit chow *ad libidum*.

On 25 May, 50 cattle tanks (800-L) were filled with 700 L of well water. To each tank I added 15 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing periphyton, phytoplankton, and zooplankton to simulate a simple aquatic community. I provided structure for the snails by placing a clay tile platform (20x20 cm tile supported by a 10x10 cm tile) in the center of each tank. I also added three predator cages to each tank. Predators that feed inside cages release chemical cues that diffuse throughout the tank without allowing the predators to kill the focal animals (Chivers and Smith 1998, Kats and Dill 1998, Tollrian and Harvell 1999). One cage, designed to house fish, was constructed from 30 x 30 cm corrugated pipe capped with fiberglass window screen on each end. The other two cages, designed to house water bugs or crayfish, were made from 10x10 cm corrugated pipe and were capped with shade cloth. I placed a shade cloth lid over each tank to prevent colonization by insects and amphibians during the experiment. On 10 June, 50 hatchling snails were added to each tank (mean mass \pm 1 SE = 19.7 ± 1.1 mg). This density (23 snails/m²) is well within natural densities of juvenile snails (J. T. Hoverman, *unpublished data*).

I designed a completely randomized experiment with 10 treatments and five replicates. The goal was to examine the responses of snails to different caged predator environments that included: 1) a no-predator control (i.e. empty cages), 2) each predator species at low density (X), 3) each predator species at high density (2X), and 4) pairwise combinations of the predator species with each predator at a low density. Based on the results from the first experiment, it was clear that snail consumption was greater with a single crayfish or fish compared to a single water bug (see *Results*). I equalized the differences in snail consumption among the predators by manipulating predator density and controlling the amount of prey given to each predator. First, I used two water bugs for every one crayfish or fish. Hence, for the three low-density predator treatments, I added one fish, one crayfish, or two water bugs to the cages. For the three high-density predator treatments, I added two fish, two crayfish, or four water bugs to the cages. The final three treatments were the three possible pairwise combinations of the predators at a low density. Second, I equalized prey consumption among predators by feeding each fish and crayfish 1 g of snail biomass while each water bug was fed 0.5 g. Therefore, predators in the low-density treatments consumed a total of 1 g of snail biomass per week while predators in the high-density treatments consumed a total of 2 g per week. Predators were fed three times per week. No uneaten snails were observed in the predator cages when the predators were fed, confirming that prey consumption was equal among predators and not affected by the presence of other predators.

Snail behavioral responses to the predator treatments were observed on 21 and 22 June (one observation per day). For each tank, I counted the number of snails that were under the tile platform (i.e. using structure) and the number of snails at the water's surface. I calculated the proportion of snails using structure and the proportion using the surface by dividing the counts by the final number of surviving snails in each tank. For each behavior, I averaged the responses over the two days for each tank and used these means as my response variables.

The experiment was terminated on 25 June before the snails had reached reproductive maturity. While the experiment lasted for just 15 d, there was, on average, a 13-fold increase in snail mass. Given this substantial increase in snail mass during the experiment, snails had sufficient opportunity to respond to the predator treatments. All surviving snails were counted and preserved in 10% formalin. Survival was greater than 90% in all treatments.

To examine treatment effects on shell characteristics, I dried 25 randomly selected snails from each tank at 80°C for 24 hrs. The dried snails were weighed to the nearest milligram and measured using digital imaging software (Optimas Co., Bothell, WA). I measured four linear shell dimensions: shell width and height and aperture width and height (see Fig. 1 in Hoverman et al. 2005). I also measured shell thickness at the leading edge of the aperture using digital calipers. After the snail was measured, I determined shell crushing-resistance by using a piston apparatus (Osenberg and Mittelbach 1989). The shell was placed on its side in a beaker and a smaller glass jar was placed flat on the shell and perpendicular to the aperture. The jar was then slowly filled with sand until the shell was crushed. The jar and sand were weighed to the nearest 0.01 g to estimate crushing resistance of the shells. While a dry shell may be weaker than a wet shell, the relative differences in shell crushing-resistance should be maintained.

4.3.1 Statistical analyses

When studying morphological plasticity, it is important to account for the allometric relationships between linear dimensions and mass (i.e. size). In my data, shell width and height, aperture width and height, and shell crush-resistance (natural-log transformed) had positive relationships with snail mass (shell thickness did not scale reliably with size). To account for size variation, I regressed the linear measurements of all individuals against their cube-root transformed mass and I saved the residuals. I then calculated the mean residual for each tank and these served as my response variables.

Since I was interested in how the predator treatments affected the multivariate response of the snails, I conducted a principal components analysis (PCA) using the five size-adjusted morphological variables, shell thickness, two behavioral responses, and mass. The first two principal components had eigenvalues greater than one and were extracted for analysis. The PC-1 and PC-2 scores were then subjected to univariate analysis of variance (ANOVA). Because PC-1 and PC-2 are uncorrelated by definition, I conducted separate ANOVAs. When univariate tests were significant, I conducted mean comparisons using Fisher's LSD test.

4.4 RESULTS

In the first experiment, I examined the mortality rates of the three predator species on small and large size classes of snails. I found a significant effect of predator species on snail mortality rates ($F_{2,6} = 80.1$, $P = 0.001$) but no effect of snail size ($F_{1,6} = 0.3$, $P = 0.629$) or the predator-by-size interaction ($F_{2,6} = 2.5$, $P = 0.166$). Averaged across snail sizes, crayfish consumed 8.67 ± 0.42 snails / hr (mean ± 1 SE), fish 7.67 ± 1.12 snails / hr, and water bugs 0.18 ± 0.03 snails / hr. Based on mean comparisons, mortality rates were similar between crayfish and fish ($P = 0.422$) but both were greater than water bugs ($P < 0.0001$)

In the second experiment, I examined how the snails altered their phenotypes in different predator environments. The PCA produced a first PC that accounted for 59% of the variation in the data and had an eigenvalue of 5.2. Positive loadings on PC-1 were associated with relatively larger shells and apertures, larger final mass, relatively weaker shells, and less use of the surface and structure. PC-2 accounted for 16% of the variation and had an eigenvalue of 1.5. Positive loadings on PC-2 were associated with thicker shells. Importantly, the variables exhibited good agreement with the extracted components (i.e. communalities > 0.6). Based on ANOVA using the scores from PC-1 and PC-2, there were significant predator effects for PC-1 ($F_{9,40} = 27.8$, $P = 0.001$) and PC-2 ($F_{9,40} = 3.6$, $P = 0.002$). Below, I summarize the responses to each predator at low and high density and then summarize the responses to combined predators (Fig. 4.1).

The first hypothesis was that each predator would induce unique responses. I tested this hypothesis by comparing snail responses among the no-predator and low-density predator treatments. Water bugs induced higher scores on PC-1 compared to snails reared in the other three treatments. In contrast, fish induced lower scores on PC-1 compared to snails reared in the other three treatments. Snails reared with crayfish were not different from snails reared without predators. There were no differences between the treatments on PC-2. In sum, low densities of water bugs and fish induced unique and opposing responses while crayfish had no effect.

I also tested this hypothesis by comparing snail responses among the no-predator and high-density predator treatments. As above, water bugs induced higher scores on PC-1 than snails reared in the other three treatments and fish induced lower scores. Snails reared with crayfish were not different from snails reared without predators. On PC-2, the no-predator, water bug, and fish treatments were not different from each other but all had lower scores

compared to the crayfish treatment. In sum, high densities of each predator induced the formation of unique responses.

The second hypothesis was that doubling the densities of conspecific predators would induce more extreme responses than the low density treatments. Doubling the density of water bugs and crayfish did not lead to significantly more extreme PC-1 or PC-2 scores. Doubling the density of fish did not affect PC-2 scores but did induce less extreme PC-1 scores.

The third hypothesis was that prey should produce intermediate responses to pairwise combinations of predators to balance the overall risk of predation. In addressing this hypothesis, the responses with combined predators must be compared to each predator alone at low and high density to assess whether snails are responding to the change in predator composition or the increase in total predator density. Below, I address this hypothesis for each of the pairwise comparisons.

I first considered the water bug-plus-crayfish treatment. On PC-1, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs at low and high densities but had significantly higher scores than snails reared with crayfish at either low or high densities. On PC-2, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs and crayfish at low densities. However, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs at high densities but had lower PC-2 scores than snails reared with crayfish at high densities. Because neither predator induced changes on PC-2 at low density (i.e. the density of each predator in the combined predator treatment) compared to the no-predator treatment, the PC-2 phenotype produced in the water bug-plus-crayfish treatment is interpreted as a lack of response to either predator and not as a risk balancing strategy. In summary, snails experiencing water bugs plus crayfish responded solely to water bugs.

I next considered the water bug-plus-fish treatment. On PC-1, snails in the water bug-plus-fish treatment were intermediate to snails reared with water bugs and fish at low densities. While snails in the water bug-plus-fish treatment had lower PC-1 scores compared to snails reared with water bugs at high densities, they were similar to snails reared with fish at high densities. On PC-2, snails in the water bug-plus-fish treatment had lower scores than snails reared without predators or with fish at low densities but were similar to snails reared with water bugs at low densities. In contrast, snails in the water bug-plus-fish treatment had lower scores

than snails reared with water bug at high densities but were similar to snails reared with fish at high densities. Since neither predator alone at low nor high density affected PC-2 scores compared to the no-predator treatment, the thinner shells produced in water bug-plus-fish treatment may be the consequence of a non-additive phenotypic response to the combined predator species. In summary, snails altered a suite of PC-1 traits when experiencing water bugs plus fish and the intermediate phenotype was biased toward the fish-induced phenotype.

Finally, I considered the crayfish-plus-fish combination. On PC-1, snails in the crayfish-plus-fish treatment were intermediate to snails reared with crayfish and fish at low densities. While snails in the crayfish-plus-fish treatment had lower PC-1 scores compared to snails reared with crayfish at high densities, they were similar to snails reared with fish at high densities. On PC-2, snails in the crayfish-plus-fish treatment were similar to snails reared with crayfish and fish at low densities. However, snails in the crayfish-plus-fish treatment were similar to snails reared with fish at high densities but had lower PC-2 scores than snails reared with crayfish at high densities. Because neither predator induced changes on PC-2 at low density compared to the no-predator treatment, the phenotype produced in the crayfish-plus-fish treatment is interpreted as a lack of response to either predator and not as a risk balancing strategy. In summary, snails altered a suite of PC-1 traits when experiencing crayfish plus fish and the intermediate phenotype was biased toward the fish-induced phenotype.

4.5 DISCUSSION

One of the major goals in community ecology is to understand predator-prey interactions within natural communities and the information gained by examining the inductive effects (i.e. non-lethal presence) of single and combined predators on prey phenotypes is a critical step toward reaching this goal. I discovered that snails display an astounding diversity of responses to different predator environments. When predator species were encountered individually, snails expressed predator-specific responses in behavior, morphology, and growth that reflected the functional differences between the predator species. When I exposed snails to different predator combinations, the resulting phenotype was biased towards one of the predators in the pair.

Below, I summarize some of the major insights gained from addressing how prey respond to more natural predator regimes.

Each of the predators induced unique responses in *Helisoma trivolvis*. Consistent with previous experiments, fish had a large impact on snail traits (Turner 1996, DeWitt et al. 2000, Turner et al. 2000). Behaviorally, snails increased their refuge use and spent more time at the water's surface with fish. Both of these behaviors can reduce encounter rates with fish that predominantly use the middle of the water column during feeding. However, an important cost of these behaviors was that the snails grew slowly. In addition to behavior and growth, fish also induced relatively smaller shells. While smaller shells had greater resistance to shell crushing, more work is necessary to determine their adaptive value. In contrast, water bugs induced phenotypes that opposed those formed with fish. For example, the snails formed relatively large shells that allow them to pull deep inside their shells (Hoverman and Relyea, *in press*). While larger shells reduce predation rates with water bugs, they come at the cost of reduced ability to resist crushing forces. Crayfish only induced changes in shell thickness in snails and only at the highest density of crayfish. Thicker shells reduce predation rates with crayfish but come at the cost of increased time to reproduction and reduced fecundity (Hoverman et al. 2005). Interestingly, thicker shells were not associated with stronger shells, possibly because shell thickness was measured at the leading edge of the aperture while shell strength was tested on the entire shell.

I found little support for the hypothesis that high predator densities induced more extreme responses than the low predator densities (although PC-1 responses to fish were slightly reduced at higher fish densities). The general lack of more extreme responses to increased water bug or fish density suggests that the phenotypic responses were maximized at low predator densities. While the prediction was not supported in water bugs or fish, the results are consistent with observations in other systems that inducible defenses often saturate at low predator densities (e.g., zooplankton, tadpoles, and bryozoans; Tollrian 1993, Harvell 1998, Van Buskirk and Arioli 2002, Relyea 2004). Nonetheless, these results are particularly striking considering the large difference in mortality risk posed by fish and water bugs (7.67 snails / hr and 0.18 snails / hr, respectively). Given the size of and mortality risk posed by fish, the mesocosms were most likely saturated with chemical cues at low fish densities. Thus, snails probably did not perceive an increase in fish density as a more risky situation. However, water bugs also induced

maximized responses at a low density despite their associated low risk of mortality. Previous experiments with water bugs have shown that snails can form defenses that reduce the risk of predation within 1 wk with limited long term reproductive costs (i.e. fecundity; Hoverman et al. 2005, Hoverman and Relyea, *in press*, Hoverman and Relyea, *unpublished data*). Accordingly, snails may have induced maximal defenses at low water bug densities because the defenses are highly effective and have limited reproductive costs. While the magnitude of antipredator defenses in prey is generally positively correlated with the amount of risk posed predators (Kusch 1995, McKelvey and Forward 1995, Anholt et al. 1996, Relyea 2001), my results and those in other systems suggest that this generalization may not hold (Relyea 2003). If prey encounter discrete environments (i.e. predators absent versus present) and there is little benefit to more extreme responses, the evolution of discrete phenotypes may be favored over continuous phenotypes (Lively 1986, Moran 1992). In this system, dose-response experiments that manipulate predation risk more precisely will be valuable in determining if the phenotypic responses of snails are continuous or discrete.

In contrast, more extreme responses were found with crayfish. For shell thickness (i.e. PC-2), I observed no responses to low densities of crayfish, but significant responses to high densities suggesting that snails are responding to either the higher predator density or the higher total amount of prey consumed. In a previous laboratory experiment using 10-L tubs, I found that one and two crayfish induced phenotypic responses in snails that were similar to each other (Hoverman et al. 2005). Importantly, the amount of prey fed to a predator (an index of chemical cue concentration) was much greater in the laboratory experiment (12 and 24 mg/L per feeding) than the current mesocosm experiment (1.4 and 2.8 mg/L per feeding). This suggests that snails require a critical threshold of chemical cue before forming phenotypic responses against crayfish. While this result was surprising given the high risk of mortality posed by crayfish (8.67 snails / hr), similar thresholds have been documented in snails and other prey species when exposed to predator cues (Snyder 1967, Brown et al. 2004, Mirza and Chivers 2004). The results demonstrate that the induction threshold for crayfish-specific defenses was greater than the threshold for the fish- and water bug-specific defenses.

The formation of predator-specific defenses in prey suggests that predators are not functionally identical entities that can be lumped together into a single mortality factor for prey (Polis and Strong 1996, Chalcraft and Resetarits 2003). Indeed, predators vary in a variety of

traits including foraging location (McPeck 1998, McIntosh and Peckarsky 1999, Schmitz and Sokol-Hessner 2002) and foraging style (Karban and Baldwin 1997, DeWitt et al. 2000) that can favor different traits in prey populations (DeWitt and Langerhans 2003, Relyea 2004). If prey experience variation in the presence of various predators and there are fitness trade-offs associated with responses to each predator, inducible defenses will be maintained over constitutive defenses (Gotthard and Nylin 1995, Kingsolver 1995a, 1995b, Dudley and Schmitt 1996). Although less frequently studied than fitness trade-offs between non-induced and predator-induced responses, a number of studies have found that functionally different predators will favor opposing phenotypic responses in prey (Tollrian and Harvell 1999, DeWitt and Langerhans 2003, Kishida and Nishimura 2005). Given that prey will face different predator species across their geographic distributions, studies that specifically address the fitness trade-offs associated with predator-specific responses to different predators will provide valuable insights into the ecology and evolution of inducible defenses.

Whereas prey in natural communities frequently encounter combinations of predators, our knowledge of inducible prey defenses comes largely from studies focused on single predator species. Prey that encounter combinations of predators must make phenotypic decisions that account for the relative risk of each predator, the frequency of encountering each predator, and the effectiveness of the defenses (McIntosh and Peckarsky 1999). By examining different predator combinations and multiple prey traits, ecologists can obtain an excellent understanding of how prey respond to more complex predator regimes. Because the predators in my experiment induced unique defenses, I expected snails to form intermediate responses that integrated their defensive strategies against the predator combination. However, I found that the responses of snails to predator combinations were strongly biased towards one predator in the combination. When snails were exposed to water bugs plus fish, they responded in a way that was clearly more similar to the fish treatment than the water bug treatment. When snails encountered fish plus crayfish, snails formed phenotypes that were similar to fish alone. Similarly, when snails encountered water bugs plus crayfish, snails formed phenotypes that were similar to water bugs alone. What are the consequences of these phenotypic decisions for prey survival?

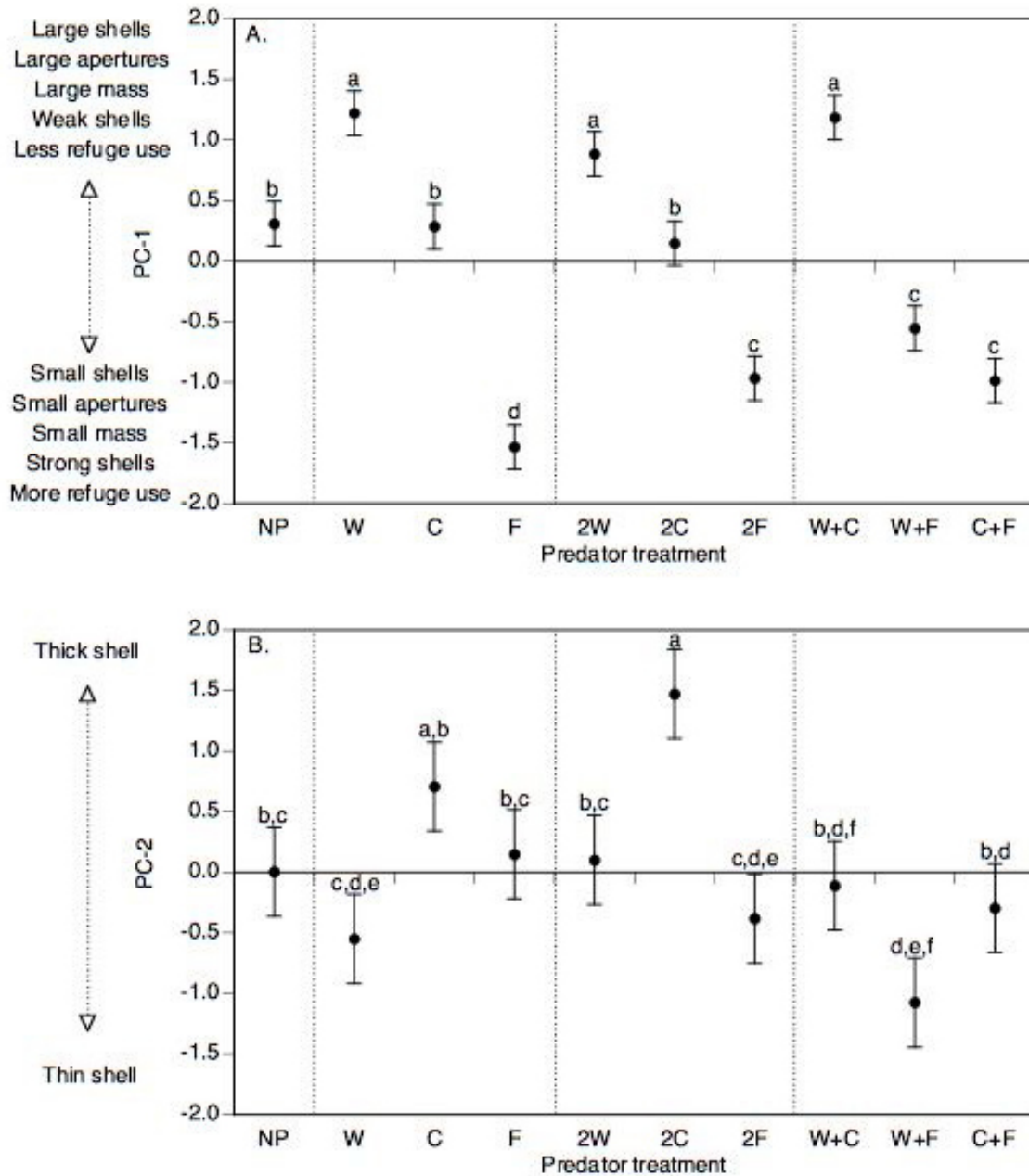
Sih et al. (1998) reviewed the literature on multiple predator effects (MPEs) and found a variety of reported effects on prey survival including risk reduction and risk enhancement. For

example, risk reduction can occur in a system when there are predator-predator interactions (e.g., intraguild predation) that alter per-capita predation rates or predator densities. In contrast, risk enhancement typically occurs when prey defenses against one predator conflict with defenses against another predator. Sih et al. (1998) concluded that risk reduction was more common than risk enhancement because many prey switch to compensatory defenses with multiple predators thereby reducing the overall risk of predation despite possible conflicting responses to different predators. By combining data on the non-lethal effects of combined predators on prey defenses and an understanding of the fitness trade-offs associated with different phenotypes, ecologists can generate predictions about prey survival with combined predators.

In the treatments containing crayfish and either fish or water bugs, snails did not respond to the crayfish. While crayfish are efficient predators on snails, it appears as though snails were unable to detect the crayfish at the low density used in the experiment. Therefore, the snails may experience high mortality from crayfish when snails focus their defensive traits (e.g., behavior and morphology) towards other predators (i.e. risk enhancement). One would also predict that snails that experience water bugs plus fish and bias their responses towards fish (e.g., relatively small shells) may experience increased predation rates by water bugs compared to when they experience water bugs alone. Risk enhancement would occur in this case because snails that possess relatively small shells are more vulnerable to attack by water bugs (Hoverman and Relyea, *in review b*). Alternatively, fish in natural communities may indirectly benefit snails by affecting water bugs in two ways. First, fish may consume water bugs thereby reducing their density and the risk that water bugs pose to snails (i.e. a density-mediated indirect interaction). Second, fish may induce behavioral changes in water bugs (e.g., foraging location) that reduce their encounter rates with snails (i.e. a trait-mediated indirect interaction). In both cases, predation rates on snail populations by water bugs would be reduced. Thus, snail populations that bias their responses towards fish when water bugs are present may, in fact, not experience an increase in predation rate. In summary, these results have provided clear predictions about how the survival of prey that express inducible defenses should be affected by predator combinations.

Predator-prey interactions are embedded within complex natural communities that can alter the predictions of experiments conducted under more simplistic conditions. This study has taken a simplified approach by examining prey responses to two different predator species encountered simultaneously. Of course, natural systems may contain more diverse predator

assemblages (i.e. more than two predator species) that include predators with different foraging techniques and locations and population densities. There is a dearth of information on the effects of more than two predators on the phenotypic responses of prey. Future work that examines how prey respond to three or more predators will be invaluable for understanding prey defensive decisions in nature. More data are also needed to address the potential for predator-predator interactions (e.g., intraguild predation) to impact the phenotypes of prey. Predator-predator interactions can affect the amount of perceived risk by prey (i.e. reduction in predator density), alter the magnitude of response to each predator (i.e. reduce the production of environmental cues), and ultimately change the outcome of multiple predator effects (Wissinger and McGrady 1993, Relyea and Yurewicz 2002, Crumrine 2005). Lastly, prey in natural communities also experience temporal variation in the predator environment (Gabriel 1999). By including natural patterns of predator variation (i.e. predator colonization and emigration), ecologists can explore how prey make phenotypic decisions that account for changes in predation risk over ontogeny. As research on multiple predators progresses, we will make significant strides towards understanding how larger communities affect the evolution and maintenance of inducible defenses.



5.0 TEMPORAL VARIATION IN PREDATION RISK: PHENOTYPES, FITNESS, AND TRAIT-MEDIATED INTERACTIONS

5.1 ABSTRACT

The study of inducible defenses has gained a great deal of insight by focusing on how different predators induce different prey defenses and, in some cases, how these defenses transmit trait-mediated indirect effects through a community. However, predators in nature are not just variable in space, but also quite variable in time. While temporal variation in predation risk has received little empirical attention, it poses a number of unique challenges for prey that are initially induced by one predator environment and subsequently must try to defend themselves against a different predator environment. Here, I examine how aquatic communities initially containing different predator environments are affected at the individual and community level by the subsequent colonization of a second predator. I exposed snails to four different caged-predator environments (no predator, fish, crayfish, or water bugs) and later exposed these different communities to predator colonization (i.e. lethal water bugs) at multiple time points. As expected, the snails responded to the caged predator environments with predator-specific behavioral and morphological defenses. When the colonizing predator was added, snails possessing the wrong phenotype attempted to induce phenotypic changes to defend themselves against the new risk. However, snails initially induced by a different predator environment often suffered high predation rates. Interestingly, in environments with snails that initially possessed the correct phenotype for the colonizing predator, an alternative prey species (tadpoles) suffered increased predation rates by the colonizing predator. Hence, temporal variation in predation risk not only challenged the snail prey to try to track this environmental variation through time by adjusting their defensive phenotypes, but also caused trait-mediated interactions between snails and the colonizing predator and between alternative prey and the colonizing predator. In

summary, I demonstrate the importance of incorporating natural patterns of environmental variation in studies addressing inducible defenses and their consequences in larger communities.

5.2 INTRODUCTION

Ecologists exploring inducible defenses are increasingly realizing that prey are not passive participants in predator-prey interactions but instead possess a variety of adaptive defenses (Karban and Baldwin 1997, Tollrian and Harvell 1999). Across their geographic ranges, prey generally experience different predator species that vary in foraging or hunting mode (Polis and Strong 1996, Chalcraft and Resetarits 2003) and, consequently, many prey form predator-specific defenses that reduce predation risk with different predators (McIntosh and Peckarsky 1999, Schmitz and Sokol-Hessner 2002, Relyea 2004). Although the emphasis for inducible defense research has been on examining how prey respond to the constant presence or absence of different predators (either alone or in combination), prey in nature also experience considerable temporal variation in predator regimes.

Temporal variation in predation risk can occur when predators colonize habitats that were previously either predator-free or contained different predator species. Theory predicts that prey should developmentally track temporal changes in the predator environment to reduce the costs of expressing the wrong phenotype in a particular environment (Gabriel 1999). Indeed, prey can express defenses throughout much of development when predators colonize predator-free habitats (i.e. wide developmental windows, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Hoverman and Relyea, *in press*). However, if predators colonize habitats that already contain other predators, prey are placed in a potentially precarious situation. If the colonizing predator favors different prey defenses than the existing predators, the subsequent phenotypic responses of prey may be constrained. Whereas we have some insight into how prey respond to combinations of predators (Relyea 2003a), we currently have no insights as to how prey adjust already induced phenotypes following colonization by a second predator.

Because of the reticulate nature of communities, inducible defenses not only affect the individuals that express the defenses but they can also affect interactions with other species in the community (termed trait-mediated indirect interactions or TMIs, Abrams 1995). Identifying

TMIs has been an important step in community ecology and TMIs driven by inducible defenses have been documented in a variety of different systems including streams (Peckarsky and McIntosh 1998), old fields (Beckerman et al. 1997), and intertidal communities (Trussell et al. 2002). However, there is a pressing need to determine whether these species interactions change under different environmental scenarios (Werner and Peacor 2003). For example, while a common trend in ecological research is to lump predators into functionally identical units (Polis and Strong 1996, Chalcraft and Reserits 2003), the existence of predator-specific defenses and consequently predator-specific TMIs suggests that predator identity can provide important insights into the transmission of TMIs because different predators have the potential to transmit predator-specific TMIs. For example, predator-specific behavioral defenses (e.g., habitat use) lead to contrasting indirect effects on resource levels in several systems (Peckarsky and McIntosh 1998, Bernot and Turner 2001, Schmitz and Suttle 2001, Schmitz et al. 2004). In sum, studies that include different predator species that induce unique prey defenses will expand our understanding of the strength and direction of TMIs.

Temporal variation in predator regimes should also affect the transmission of TMIs. A TMII may occur if the presence of the first predator prevents the expression of an appropriate defense against the colonizing predator and thereby causes increased prey mortality (i.e. predator facilitation, Charnov et al. 1976, Soluk and Collins 1988). Unfortunately, because we lack data on whether prey can respond to a colonizing predator when other inducing predators are already present, we do not know whether existing predators can cause TMIs between the prey and a colonizing predator (i.e. altered predation rates). If so, then a colonizing predator has the potential to affect other species in a community including alternative prey. For example, if a given prey species is well defended against the colonizing predator, predation pressure may increase on other prey species. In contrast, if a given prey species is poorly defended against the colonizing predator, predation pressure may decrease on other prey species (Abrams 1987). In such cases, changes in predation rates would be mediated by the defensive traits expressed by the focal prey rather than by the densities of the focal prey (i.e. a TMII). While prey nutritional quality, handling time, and abundance play important roles in understanding the prey preferences of predators (Murdoch 1969, van Baalen et al. 2001), the anti-predator defenses of prey within the community may be equally as important (Abrams 1987, Bolker et al. 2003). Thus, we need to determine how temporal variation in predation regimes can affect the transmission of TMIs.

I examined the impact of predator identity and temporal variation in predator regime on prey defenses and TMIs using simple freshwater communities composed of two herbivores that share an algal resource (planorbid snails, *Helisoma trivolvis*, and green frog tadpoles, *Rana clamitans*) and one of three common predators (pumpkinseed sunfish, *Lepomis gibbosus*, crayfish, *Orconectes rusticus*, and water bugs, *Belostoma flumineum*). Previous work has demonstrated that freshwater snails in the laboratory exhibit predator-specific behavioral and morphological defenses in response to cues from water bugs and crayfish (Hoverman et al. 2005) and that these defenses have relatively wide developmental windows combined with the ability to reverse defensive phenotypes early in ontogeny (Hoverman and Relyea, *in press*). Whereas many tadpoles also have predator-specific defenses (Relyea 2001a), previous work has shown that tadpoles do not respond to predators consuming snails (Schoeppner and Relyea 2005) and green frog tadpoles do not respond to predatory water bugs eating tadpoles (Relyea 2001b). Thus, I could examine whether tadpole growth and survival were indirectly affected by caged predator treatments that solely affect snail phenotypes. To incorporate temporal variation, I simulated the migration of predators using lethal (i.e. uncaged) water bugs that naturally migrate into freshwater communities. My specific questions were the following: 1) How do the three predators affect the behavior, morphology, and growth of snails under mesocosm conditions? 2) Do the predator-specific defenses of snails indirectly affect tadpole growth? 2) Does the initial induction by different species of predators affect the interaction between snails and colonizing water bugs?, and 3) Do the predator-specific defenses of snails indirectly affect tadpole predation by colonizing water bugs?

5.3 METHODS

I examined the effects of caged predators and lethal water bug colonization on a simple aquatic community by conducting a mesocosm experiment at the University of Pittsburgh's Aquatic Research Facility in Linesville, PA. On 27 March 2004, I collected 350 adult snails from a local pond and placed 25 into each of 14 pools filled with 100 L of well water to oviposit. Egg deposition began in April and continued until May, at which time the adults were removed from the pools. Snails began hatching on 9 May and were fed rabbit chow *ad libitum* until the start of

the experiment. A mixture of four green frog (*Rana clamitans*) egg masses were collected from a nearby pond on 18 July and placed into four pools. Tadpoles began hatching after one week and were fed rabbit chow *ad libitum*.

On 19 July, 48 cattle tanks (800-L) were filled with 700 L of well water. To each tank, I added 15 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing periphyton, phytoplankton, and zooplankton from nearby ponds to simulate a simple aquatic community. A single clay tile platform (20x20 cm tile supported by a 10x10 cm tile) was placed in the center of each tank to serve as artificial structure. I also added three predator cages to each tank. One cage, designed to house fish, was constructed from 30x30 cm corrugated pipe capped with fiberglass window screen on each end. The other two cages, designed to house crayfish and water bugs, were made from 10x10 cm corrugated pipe capped with shade cloth. I placed a shade cloth lid over each tank to prevent colonization by insects and amphibians during the experiment. On 30 July, I added 50 tadpoles (mean mass ± 1 SE = 12 ± 4 mg.) and 50 juvenile snails to each tank (73 ± 4 mg). These densities (23 snails or tadpoles/m²) are within natural densities for the two species (J.T. Hoverman, *unpublished data*).

I designed a completely randomized experiment with 12 treatments and 4 replicates. The experiment was a factorial combination of caged predators and lethal water bug colonization. The caged predator treatments were the following: 1) no predator, 2) two water bugs, 3) two crayfish, and 4) one fish. Caged predators emit water-borne chemical cues, which provide the opportunity to examine trait induction without a reduction in target prey density (Chivers and Smith 1998). All caged predators were fed three times per week. To equalize the total amount of prey biomass consumed, each caged fish was fed 1 g of snail biomass while each caged water bug and crayfish was fed 0.5 g of snail biomass at each feeding.

I examined temporal variation in predation risk using lethal (i.e. uncaged) water bugs. This predator is common in freshwater systems but displays fluctuations in population density (J.T. Hoverman, *unpublished data*). Water bug densities are generally low in May (< 0.5 adults/m²) but can increase dramatically by July (e.g., 14 adults/m²) due to reproduction and migration from permanent over-wintering ponds to more ephemeral ponds. The lethal water bug treatments were the following: 1) no water bug colonization, 2) early colonization by two water bugs (on day 10), and 3) late colonization by two water bugs (on day 20).

Tadpole and snail behavior were observed on day nine of the experiment before the initiation of the water bug colonization treatments. I assessed tadpole behavior by quantifying the number of tadpoles that could be observed and the proportion of observed tadpoles that were moving (i.e. active). For each tank, I conducted four observations and calculated the mean number of tadpoles seen and the mean percent activity to serve as my response variables. I assessed snail behavior by counting the number of snails that were under the tile platform (i.e. using structure) and the number of snails at the water's surface. I calculated the proportion of snails using structure and the surface by dividing the counts by 50 (i.e. the initial number of snails added to each tank). These proportions were used as my two response variables for snail behavior. Since the observations required that I lift the tile platform and disturb the snails, I only conducted a single observation. These protocols have been used successfully in previous experiments examining tadpole and snail behavior (Turner 1996, Relyea 2001b).

The experiment was terminated on day 28 and all surviving snails and tadpoles were counted to assess survival and preserved in 10% formalin to later quantify mass and morphology. The tadpoles and snails from each tank were weighed and then mean tadpole and snail mass were used as the mass response variables. Because one of my major goals was to examine treatment effects on snail shell shape, 20 randomly selected snails from each tank were weighed to the nearest milligram and measured for shell height and width using digital imaging software (Optimas Co., Bothell, WA). I also measured the shell thickness (at the leading edge of the aperture) of each snail using digital calipers.

When studying morphological plasticity, it is important to account for the allometric relationships between linear dimensions and mass (i.e. size). In my data, shell width and height positively covaried with snail mass (cube-root transformed to make the relationship linear). Thus, I used analysis of covariance (ANCOVA) with mass as the covariate to correct for size (Hoverman et al. 2005). A critical assumption in the ANCOVA procedure is that the treatments share a common slope of their regression lines and the data met this assumption. From the ANCOVA, I used the mass-adjusted treatment means and residuals from the within-treatment regressions to calculate each individual's mass-adjusted value. For each morphological trait, I then calculated the mean mass-adjusted shell dimensions for each experimental unit and used these means as my morphological response variables. Shell thickness did not covary with mass even after various transformations, which is consistent with previous work in this system

(Hoverman et al. 2005, Hoverman and Relyea, *in press*). Thus, I simply calculated the mean shell thickness for the snails from each tank and this served as my shell thickness response variable.

I conducted two analyses with the data. First, I used a multivariate analysis of variance (MANOVA) to analyze the effect of caged predators on tadpole behavior (number seen and percent active) and snail behavior (percent at the water surface and percent using structure) on day 9, which was just prior to applying the water bug colonization treatments. Second, I used a factorial MANOVA to examine the effects of caged predators, water bug colonization, and their interaction on snail morphology, snail and tadpole growth, and snail and tadpole survival. Prior to the analysis, snail and tadpole mass were arcsine- and log-transformed, respectively whereas snail and tadpole survival were arcsine-transformed. For all the analyses, significant multivariate effects were followed by univariate tests. When univariate tests were significant, I conducted mean comparisons using Fisher's LSD test.

5.4 RESULTS

5.4.1 Prey behavior

In the first analysis, I examined the effects of caged predators on the behavior of tadpoles and snails and found a significant multivariate effect (Wilks' $\lambda_{12,109} = 9.9$, $P < 0.001$). While caged predators did not affect tadpole behavior (univariate $P \geq 0.194$; Fig. 5.1A), the predators did affect snail behavior (univariate $P \leq 0.001$; Fig 5.1B). Compared to the no-predator treatment, caged water bugs had no effect on snail behavior, caged crayfish had no effect on the use of the surface but induced 10% more snails to move under structure, and caged fish induced 20% greater use of the surface and 48% greater use of structure.

5.4.2 Snail morphology

In the second analysis, I examined the treatment effects on snail morphology, snail and tadpole mass, and snail and tadpole survival. There was a significant multivariate effect of caged predators, lethal water bug colonization, and their interaction. Below, I examine each of the univariate response variables.

Shell height was affected by caged predators but not by water bug colonization or the interaction (Table 5.1, Fig. 5.2A). Compared to the no-predator treatment, snails reared with crayfish and water bugs were not different while snails reared with fish had 3% lower shells. Snails reared with water bugs also had higher shells than snails reared with fish but snails reared with crayfish or fish did not differ.

Shell width was affected by caged predators, water bug colonization, and their interaction (Table 5.1, Fig. 5.2B). To determine the patterns of defenses induced by each of the caged predators, I can examine the treatments lacking any colonization. In these treatments, caged water bugs induced 4-6% wider shells than the other three treatments and the other three did not differ from each other. To determine the patterns of induced defenses due to subsequent colonization, I can look within each caged-predator environment. Within the no-predator environments, early and late colonization by water bugs induced 8-9% wider shells than the no-colonization treatment. Within the caged-water bug environments, shell width was not affected by early or late colonization. Within the caged-crayfish environments, late colonization induced marginally wider shells whereas early colonization induced 6% wider shells compared to the no-colonization treatment. Within the caged-fish environments, late- and early- colonization induced 4-6% wider shells than the no-colonization treatment. In summary, cues from caged water bugs induced wider shells than the other three caged-predator environments and the subsequent colonization by water bugs induced the latter three predator environments to form wider shells.

Caged predators, water bug colonization, and their interaction also affected shell thickness (Table 5.1, Fig. 5.2C). To determine the patterns of defenses induced by each of the caged predators, I can again examine the treatments lacking colonization. Compared to the no-predator treatment, caged water bugs induced no change, caged crayfish induced 67% thicker shells, and caged fish induced 85% thinner shells. To determine the patterns of induced defenses

due to subsequent colonization, I can look within each caged-predator environment. Within the no-predator environments, shell thickness in the no-colonization and early-colonization treatments was 88% thicker than the late-colonization treatment but there was no difference between no colonization and early colonization. Within the caged-water bug environments, shell thickness was not affected by colonization. Within the caged-crayfish environments, shell thickness in the no-colonization and early-colonization treatments were 76-80% thicker than the late-colonization treatment; there was no difference between no-colonization and early-colonization. Within the caged-fish environments, shell thickness was not affected by colonization. In summary, caged crayfish induced thick shells, caged fish induced thin shells, and water bugs had no effect; the colonization by lethal water bugs in the no-predator and caged-crayfish environments for shorter durations (i.e. late colonization) caused large reductions in shell thickness whereas colonization for longer durations (i.e. early colonization) caused a rebound to thicker shells.

5.4.3 Prey mass

Snail mass was affected by caged predators but not by water bug colonization or the interaction (Table 5.1; Fig. 5.3). Compared to the no-predator environment, caged water bugs had no effect, caged crayfish induced 15% larger mass, and caged fish induced 22% smaller mass. Snails reared with caged water bugs and caged fish were 12% and 33% smaller, respectively, than snails reared with caged crayfish. Snails reared with caged fish were 24% smaller than snails reared with caged water bugs.

Tadpole mass was affected by caged predators but not water bug colonization or their interaction (Table 5.1; Fig. 5.3). Compared to the no-predator environment, caged water bugs had no effect while caged crayfish and caged fish induced 31 and 40% larger mass, respectively. There were no differences among the three caged predator treatments.

5.4.4 Prey survival

Caged predators, water bug colonization, and the interaction affected snail survival (Table 1, Fig. 4). By examining survivorship within each predator environment, I can compare survival

without lethal water bugs to survival when lethal water bugs were present for either a relatively short (i.e. late colonization) or long (i.e. early colonization) period of time. Within all of the caged-predator environments, snail survival declined from no colonization to early colonization.

By examining survivorship within each colonization treatment, I can assess how existing defenses affected snail survival against colonizing water bugs. When there was no colonization, survival was high across all caged-predator environments (mean = 98%). When there was late colonization, there was only one significant comparison; snails reared with caged water bugs survived 10% better than snails reared with caged fish. However, when there was early colonization, snails reared in no-predator environments experienced survival that was similar to snails reared in caged-water bug environments but 14% and 36% higher than snails reared in caged-crayfish and caged-fish environments, respectively. In summary, snails survived water bug colonization the best when they were previously induced by water bugs or no predators and the worst when they were previously induced by fish.

Tadpole survival exhibited no main effect of caged predators but there was an effect of water bug colonization and a predator-by-colonization interaction (Table 5.1, Fig. 5.4). By comparing survivorship within each predator environment, I can assess the impact of lethal water bugs on tadpole survival. In the no-predator environments, an increased duration of lethal water bugs (from no colonization to early colonization) had no effect on tadpole survival. However, the other three caged-predator environments all experienced a decline in tadpole survival.

By comparing survivorship within each colonization treatment, I can assess how existing snail defenses indirectly affected tadpole survival against colonizing water bugs. Within the no-colonization treatments, tadpoles living with non-induced snails experienced 9-10% lower survival than tadpoles living with water bug- and crayfish-induced snails but similar survival as tadpoles living with fish-induced snails. Within the late colonization treatments, tadpole survival was similar among caged-predator environments. However, within the early colonization treatments, tadpoles living with non-induced snails experienced 24% higher survival than tadpoles living with water bug-induced snails but similar survival as tadpoles living with crayfish- and fish-induced snails. In summary, tadpole survival frequently declined with longer exposures to lethal water bugs, but the amount of survival depended upon the caged-predator environment in which the tadpoles were embedded. As a result, tadpoles living with water bug-induced snails experienced the lowest survival.

5.5 DISCUSSION

The results of this study demonstrate that spatial and temporal variation in predation regime via predator identity and predator colonization can affect the phenotypes expressed by prey and the subsequent TMIs in a community. Consistent with my previous work with water bugs and crayfish in the laboratory (Hoverman et al. 2005), I found that *Helisoma* snails formed predator-specific defenses that reflect the differences in how the predators consume snails. Based on selection experiments (Hoverman and Relyea, *unpublished data*), these responses appear to be adaptive. Water bugs induced snails to form wider shells that allow snails to pull deep inside the shell and escape the probing stylet of the water bug. While wider shells reduce the risk of predation by water bugs, they come at the cost of delayed reproduction. Crayfish induced weak habitat shifts (10% increased use of structure) and induced snails to form thicker shells. Thicker shells are more resistant to aperture chipping by crayfish, but the defense comes at the cost of reduced fecundity. Fish strongly induced snails to avoid the middle of the water column. This movement should reduce encounter rates with fish but it clearly comes at the cost of substantially reduced growth. Overall, these results parallel those in other systems in which prey form predator-specific defenses as a consequence of the functional differences among their predators (Karbon and Baldwin 1997, Tollrian and Dodson 1999, Relyea 2001b, DeWitt et al. 2000). These functional differences among predators can lead to fitness trade-offs for different phenotypes, which should favor the evolution of inducible rather than constitutive defenses (Kingsolver 1995, Dudley and Schmitt 1996).

This experiment provided a unique opportunity to examine how temporal variation in the predator regime affects prey phenotypes that were initially exposed to different predators and the consequences of those phenotypes for prey survival. When water bugs colonized, they had no effect on the shell morphology of snails that were previously exposed to caged water bugs, suggesting that these snails were already maximally induced. However, when water bugs colonized tanks containing no predators or caged crayfish, the snails subsequently exhibited relatively wider shells. Although the wider shells could be the consequence of either selection for or induction of wider shells by lethal water bugs, induction likely played the dominant role because the magnitude of mortality was similar in the no-predator and caged-water bug

environments (yet only the former experienced an increase in shell width following colonization).

In previous work on *Helisoma* snails, I found that when non-lethal (i.e. caged) water bugs are added to predator-free habitats the snails quickly develop wider shells, but these wider shells are initially built using very thin shell material. However, within 3 wks, the snails are able to thicken their shells to resemble those snails that had continuous exposure to caged water bugs. (Hoverman and Relyea, *in press*). Interestingly, I saw a similar pattern when lethal water bugs were added to either no-predator or caged-crayfish environments (Fig. 2); in both cases, the short-duration addition of lethal water bugs (i.e. late colonization) was associated with very thin shells whereas the long-duration addition of water bugs (i.e. early colonization) was associated with a shell thickness that resembled those snails induced by water bugs throughout the entire experiment. Hence, although water bugs and crayfish differ functionally (i.e. shell invader versus shell chipper), snails appear to be capable of expressing an appropriate defense against both predators (i.e. wider and thicker shells). To do so, however, the snails require a substantial amount of time (~3 wks) and during that time the snails would be particularly vulnerable to both predators since they are inadequately defended against both.

The growth of the snails differed among the predator environments in ways that suggested a combination of predatory fear effects and fertilization effects. In the majority of predator induction studies, prey express their defenses at the cost of reduced growth or fecundity because there is a reduction of foraging behavior (Lima and Dill 1998). Thus, it was not surprising that the species of predator that induced the greatest behavioral responses in the snails (i.e. fish) also induced a large reduction in snail growth. At the same time, however, caged crayfish induced an increase in snail mass. Snails can reach a size refuge from crayfish by delaying reproduction and allocating resources to overall size and shell thickness. However, this strategy comes at the cost of reduced fecundity (Hoverman et al. 2005). Alternatively, increases in prey growth with predators present (but no change in prey density) are typically attributed to the nutrients that predators add to the water via prey digestion which fertilize algal growth (i.e. consumer-mediated nutrient recycling, Vanni and Layne 1997). Indeed, predators can alter the abundance and structure of producer communities via increased nutrient inputs or altered nutrient stoichiometry (McCollum et al. 1998, Elser and Urabe 1999). Thus, the final impact on prey mass should reflect the growth decreasing effects of fear opposed by the growth increasing

effects of fertilization. Given that all three of the caged predators digested the same mass of prey, one might expect all three predators to cause similar fertilization. If so, then the high rate of snail growth with crayfish would reflect relatively weak fear effects, the moderate rate of snail growth with water bugs would reflect moderate fear effects, and the low rate of snail growth with fish would reflect strong fear effects.

If the snails and tadpoles were competing for periphyton in the mesocosms, I would expect to see any predator-induced negative effects on snail mass to be associated with indirect positive effects on tadpole mass. In this experiment, I found that the tadpoles increased growth in both the caged-crayfish and caged-fish environments. As expected from previous research, tadpoles did not change their behavior when the caged predators were fed snails, either because tadpoles do not recognize the cues generated by predation on snails or because predation on snails communicates a low level of predation risk to tadpoles (Lefcort et al. 1999, Schoeppner and Relyea 2005, Persons et al. 2001). Therefore, the changes in tadpole growth were not due to direct changes in any tadpole traits.

There was no suggestion that the tadpoles and snails were competing, making a TMII on tadpole growth difficult to witness. The strongest evidence for no competitive effects comes from a lack of increased growth in either snails or tadpoles following the addition of lethal water bugs, despite experiencing up to 50% snail mortality and 40% tadpole mortality. Moreover, past studies have shown that tadpoles and snails can feed on different groups of periphytic algae (diatoms and green algae, respectively; Werner and Peacor 2006). Thus, at the densities employed in the study, the low diet overlap may have precluded any competitive effects. Thus, in the case of caged-crayfish environments, the most parsimonious explanation for the increase in tadpole growth is that both the snails and the tadpoles were benefiting from the fertilization effect of crayfish digestion. In the case of caged-fish environments, the most parsimonious explanation for the increase in tadpole growth is that the tadpoles were benefiting from the fertilization effect of fish digestion as well as (perhaps) the 22% decline in snail growth that should increase periphyton abundance even more (Bernot and Turner 2001). In summary, while the tadpoles did experience changes in growth due to the presence of snail-specific predator cues, there is little evidence that these growth changes reflect a TMII.

There was good evidence that the presence of caged-predators produced a TMII in which the initial induction by different predator environments caused different predation rates on snails

when lethal water bugs colonized. I found that snails living in no-predator and caged-water bug environments had similar survival following early water bug colonization, but snails living with caged-crayfish and caged-fish suffered 14-36% higher predation. These changes in predation rates are tied to the defenses that the snails possessed at the time of the colonization. Based on inferences from the final phenotypes, fish-induced snails had narrow shells and smaller overall mass, making these snails particularly vulnerable to a water bug predator that preferentially kills such phenotypes (Hoverman and Relyea, *unpublished data*). Crayfish-induced snails, on the other hand, had greater mass but they also possessed a poor defensive morphology against water bugs, making them somewhat less vulnerable. Water bug-induced snails would have had the most appropriate defense against colonizing water bugs and, as expected, experienced the lowest rates of predation. Why the non-induced snails would have similar survival as the water bug-induced snails after early colonization is unclear, although the lack of behavioral responses by the non-induced snails may have allowed them to respond more rapidly to the colonizing water bugs and quickly become invulnerable. In general, these results demonstrate that the initial occurrence of one species of predator can facilitate predation by a second predator that colonizes at a later date.

In communities with multiple prey species, the level of defense expressed by different prey species can affect the predation rates on alternative prey (i.e. a TMII; Abrams 1987, Matsuda et al. 1994, Bolker et al. 2003). In this experiment, it was clear that different caged-predator environments indirectly affected the predation rates of colonizing water bugs on tadpoles. For example, when there was no water bug colonization, I found that tadpoles in the caged-water bug environment survived a bit better (+10%) than tadpoles in the no-predator environment. However, if there was early colonization by water bugs, tadpoles in the caged-water bug environment survived much worse (-24%). The tadpoles did not behave differently nor have any difference in mass between these two treatments, suggesting that the difference in predation rate was driven by the fact that snails in the water bug-environment had an adaptive defense at the time of water bug colonization and this caused increased predation on tadpoles. However, in potential conflict with this mechanism is the fact that the caged-predator environments experienced similar levels of tadpole death with early colonization of water bugs, suggesting that snail morphological defenses were perhaps not the only cause. One alternative explanation is that the caged-predator environments affected the predatory behavior of the

colonizing water bugs. Interestingly, water bugs consumed approximately twice as many total prey (within the early colonization treatment) when there were caged predators present than when no predators were present. Increasing the density of predators in the tanks (detectable via visual or chemical cues) may have caused an increase in water bug foraging (a common response to competition by animals; Stephens and Krebs 1986, Relyea 2002). Because the snails in caged-water bug environments were well defended against colonizing water bugs, increased foraging activity by the colonizing water bugs would have been directed towards the tadpoles in the caged-water bug environments. In contrast, since snails in caged-fish and caged-crayfish environments were poorly defended against colonizing water bugs, increased foraging activity by the colonizing water bugs would have been directed towards both the snails and tadpoles. If this scenario were correct, tadpole predation rates would be mediated by a combination of altered predator foraging and prey morphological defenses (i.e. two types of TMIs). As previous authors have suggested, many more studies are needed to determine whether changes in predation rates are mediated by prey traits or by other predators in the system (Lima 2002).

5.5.1 Conclusions

Temporal variation is a fundamental component of natural ecosystems and has been used to study a variety of questions concerning phenotypic plasticity (Piersma and Lindström 1997, Weinig and Delph 2001, Miner and Vonesh 2004). Surprisingly, temporal variation has only recently been incorporated into studies of predator-prey interactions (Kuhlmann et al. 1999, Tollrian and Dodson 1999, Van Buskirk 2002, Hoverman and Relyea, *in press*). Since predator regimes can commonly fluctuate during prey development, reacting to this variation is probably a common challenge for organisms in nature. If prey frequently encounter temporal variation and exhibit poor phenotype-environment matches, phenotypic plasticity may be selected against in the population (Padilla and Adolf 1996). However, if prey can respond to temporal variation with appropriate phenotype-environment matches, we should witness wide developmental windows of inducibility and relatively rapid inducibility of prey defenses (Gabriel 1999, Relyea 2003b, Hoverman and Relyea, *in press*).

Temporal variation in predation not only affects the individual, but also can affect the larger ecological community via trait-mediated indirect effects. Recently, researchers have

addressed the importance of inducible defenses within more complex communities where the potential for density- and trait-mediated indirect interactions is immense. While the vast majority of theoretical and empirical work has focused on behaviorally-mediated indirect interactions (Bolker et al. 2003, Werner and Peacor 2003, Schmitz et al. 2004), it is clear that indirect interactions can be mediated by changes in prey morphology and life history (Relyea 2000, Trussell et al. 2002). By incorporating a diversity of traits, we can begin to determine the importance of trait type in the strength and transmission of TMIs. My results make it clear that defensive responses to one predator can facilitate the predation rate of a second, colonizing predator on both the focal prey and on alternative prey. Moreover, the behavior of predators and the morphology of prey may interact to impact the survival of alternative prey in the system when predator densities change over time. Collectively, this suggests that to understand the evolution and ecology of inducible defenses including how they impact natural communities we will need to incorporate both the spatial and temporal variation that we observe in nature.

Table 5.1 Results of a MANOVA on the effects of different caged predator treatments and water bug colonization on snail morphology, snail and tadpole mass, and snail and tadpole survival (A). Univariate tests (P-values) are shown for both main effects and their interaction (B).

A. Multivariate		df	F	P
Caged predator		21,86	7.9	<0.001
Water bug colonization		14,60	19.8	<0.001
Caged predator * colonization		42,144	3.2	<0.001
B. Univariate				
	Caged predator	Colonization	Caged predator * colonization	
Shell height	0.009	0.450	0.120	
Shell width	<0.001	0.001	0.023	
Shell thickness	<0.001	<0.001	<0.001	
Snail mass	<0.001	0.111	0.753	
Tadpole mass	0.025	0.103	0.923	
Snail survival	<0.001	<0.001	0.027	
Tadpole survival	0.349	<0.001	0.020	

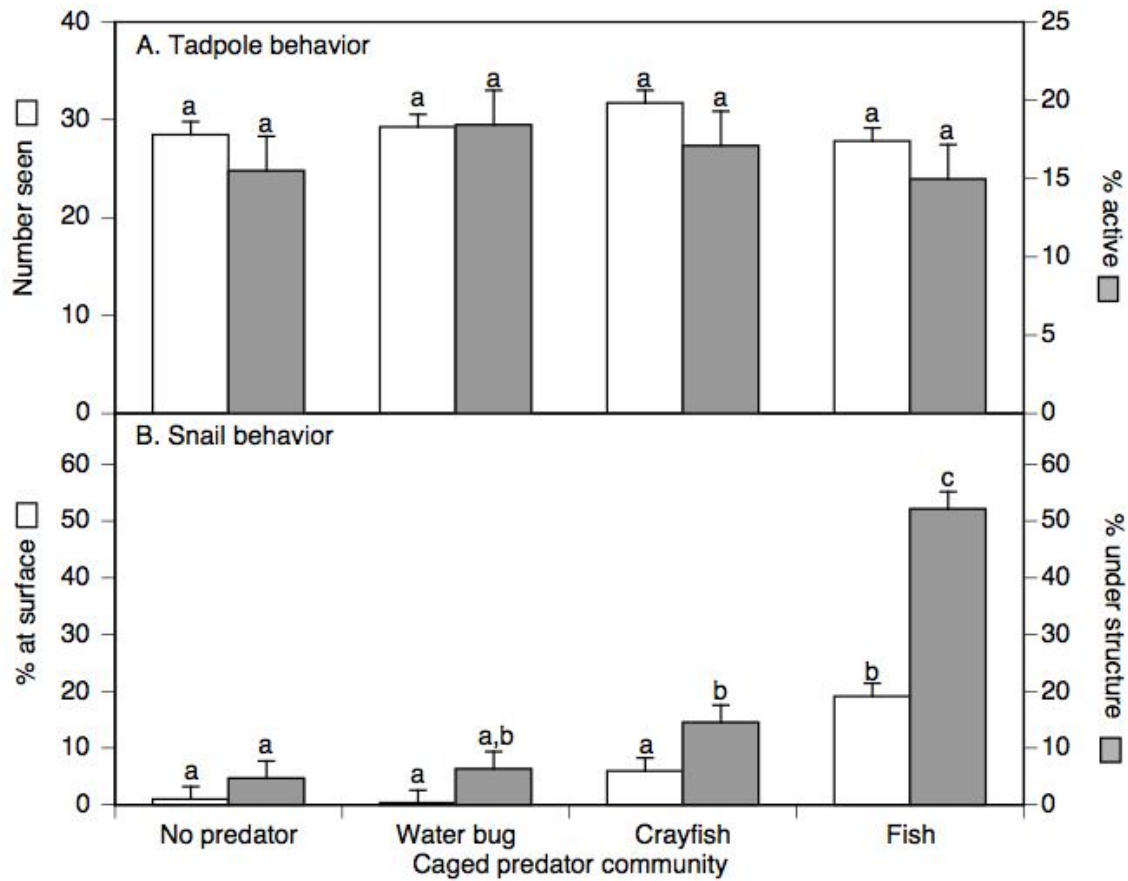


Figure 5.1 The effects of different caged predator treatments on: A) green frog tadpole behavior (the number of tadpoles seen and percent activity) and B) snail behavior (the use of surface and structure). Observations were taken prior to the colonization of lethal water bugs. Data are least-squares means \pm 1 SE. For each trait, treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($P > 0.05$).

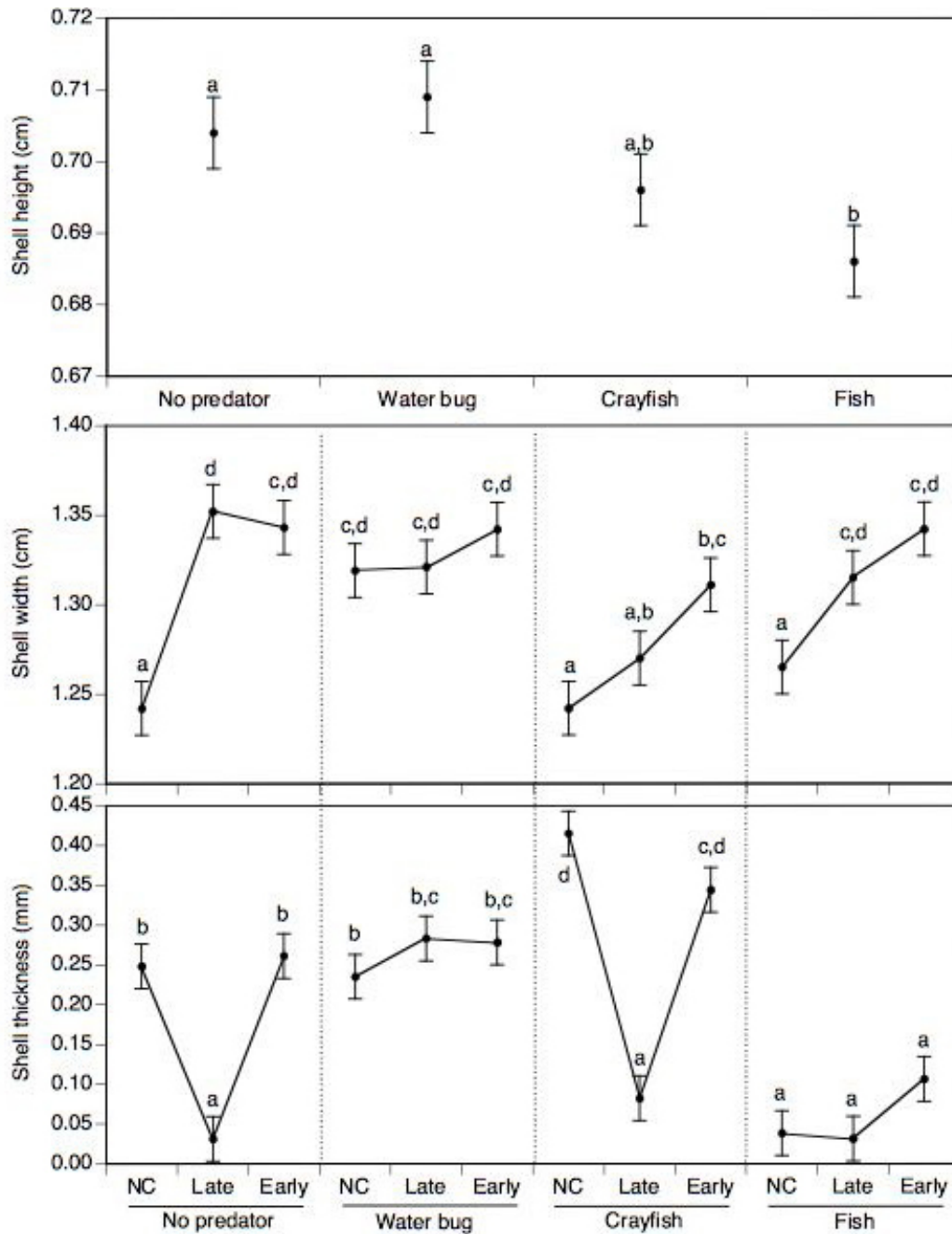


Figure 5.2 The effects of different caged predator treatments and water bug colonization on the final shell height, width, and thickness of snails. The lethal water bug colonization treatments were either no colonization, early colonization (i.e. two lethal water bugs added at day 10), or late colonization (i.e. two lethal water bugs added at day 20). Shell height and width were corrected for size by regressing the linear dimensions against cube-root transformed mass (using analyses of covariance) and saving the residuals for each tank. The data for shell height are averaged over water bug colonization treatments (see text for details). Data are least-squares means \pm 1 SE. Treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($P > 0.05$).

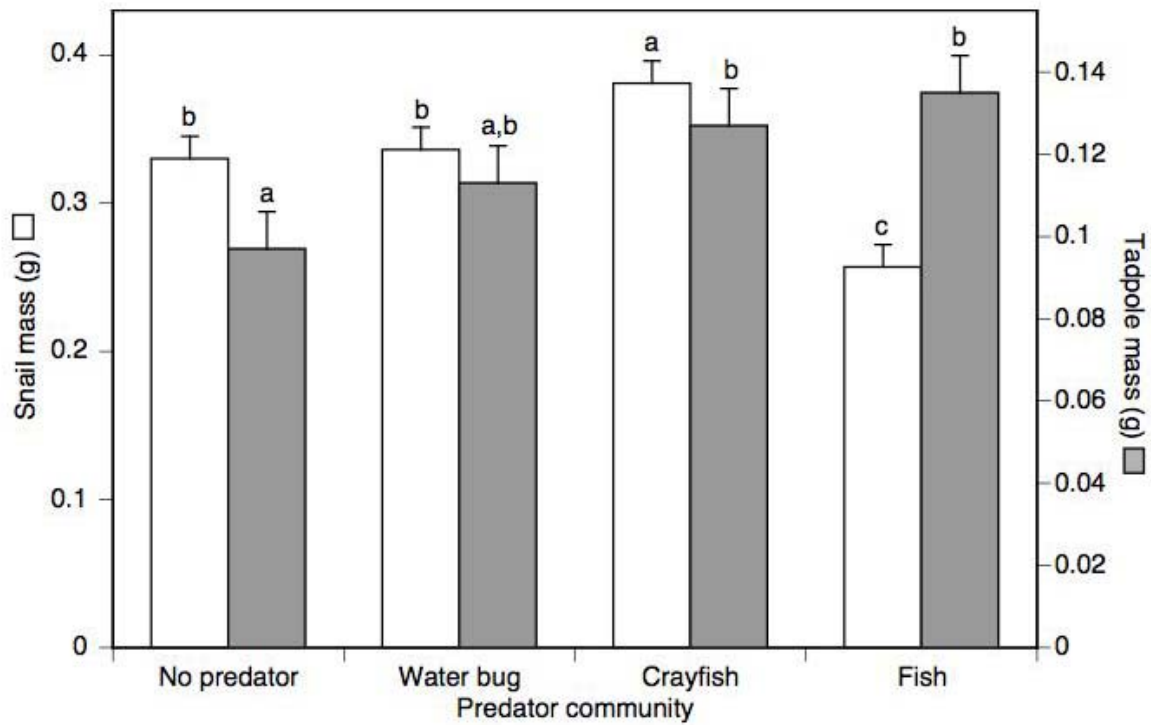


Figure 5.3 The effects of different caged predator treatments on the final mass of tadpoles and snails. Data are least-squares means \pm 1 SE. For both variables, treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($P > 0.05$).

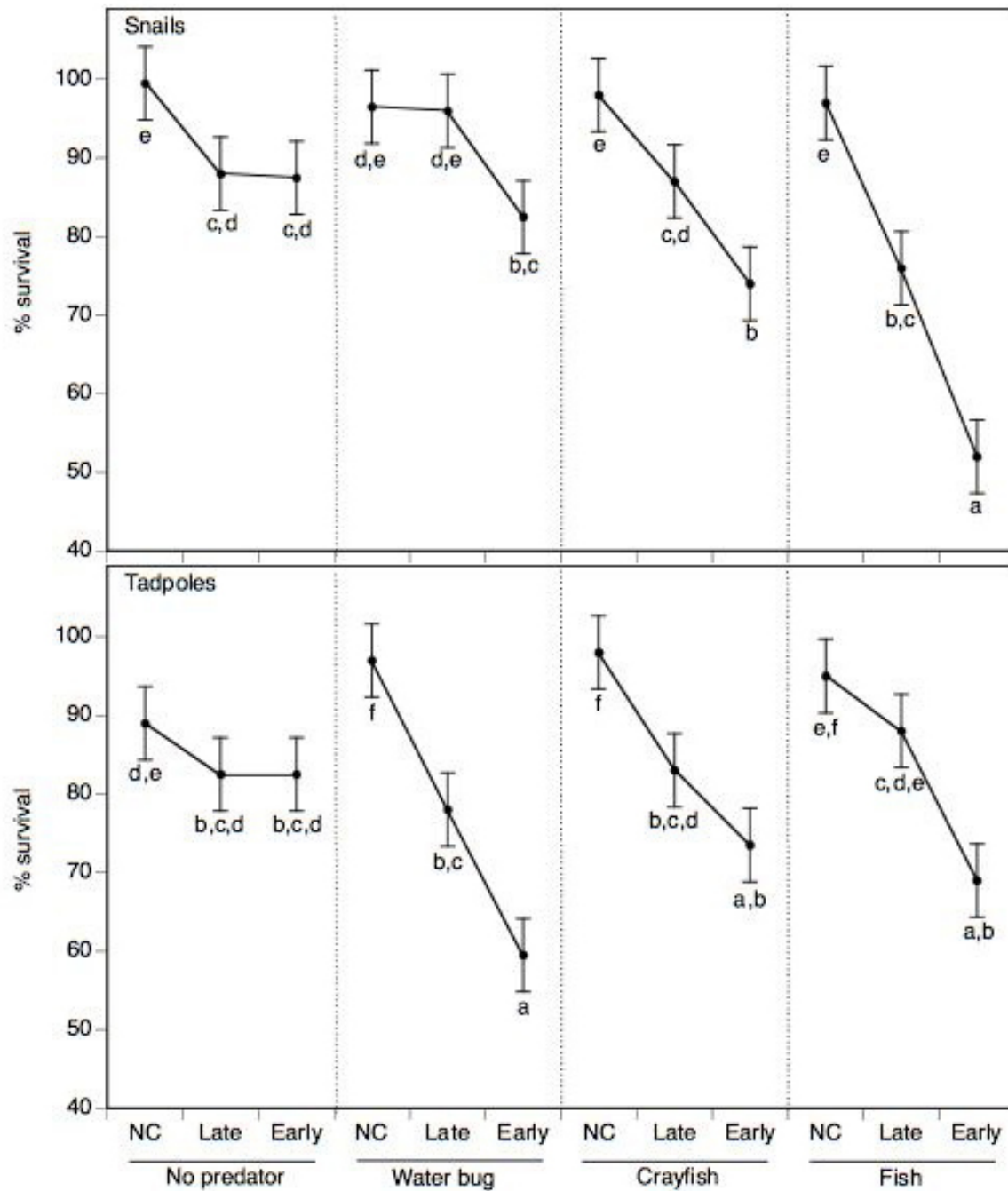


Figure 5.4 The effects of different caged predator treatments and water bug colonization on snail and tadpole survival. Data are least-squares means \pm 1 SE. Treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($P > 0.05$).

6.0 CONCLUSIONS AND FUTURE DIRECTIONS

Environmental variation can dramatically affect phenotypic expression both within and between populations. This dissertation reinforces the fact that some environmentally induced phenotypes are not simply developmental noise rather they can be adaptive options that improve fitness in the face of environmental variability. More specifically, I demonstrated that the inducible defenses of prey have a benefit of reducing predation rates with a given predator but lead to increased susceptibility to other functionally different predators and come at the cost of delayed reproduction, reduced fecundity, or slower growth. Below, I will discuss some of the major insights obtained from my work as well as some future directions for researchers interested in phenotypic plasticity.

While there has been a gradual accumulation of studies documenting phenotypic plasticity in a variety of systems, there are far fewer studies that rigorously test the adaptive plasticity hypothesis. The hypothesis posits that plasticity is favored because of fitness trade-offs associated with the induced phenotypes in alternative environments and that there are divergent selective pressures on the traits exhibiting plasticity. In predator-prey systems, several studies have identified the fitness benefits of inducible defenses (i.e. reduced predation rates) but few have identified the fitness costs (Van Buskirk and Relyea 1998, Tollrian and Dodson 1999, Tollrian and Harvell 1999, Van Buskirk and Schmidt 2000, Kishida and Nishimura 2005, Benard 2006). In addition, there have been relatively few studies that have documented that selection favors plasticity (i.e. divergent selection on the traits exhibiting plasticity; Baldwin et al. 1990, Spitze 1992, McCollum and Van Buskirk 1996, Baldwin 1998, Van Buskirk and Relyea 1998, Van Buskirk and Schmidt 2001). Without a test of the adaptive plasticity hypothesis, it is unclear whether plasticity was the result of natural selection or simply a consequence of the environment's effect on phenotypic expression (Doughty and Reznick 2004). While identifying the traits that exhibit plasticity is an important step in research programs exploring

environmentally induced phenotypes, we need more studies that take the next step by definitively testing the adaptive plasticity hypothesis. With this data, we can begin to address questions concerning the ecological and evolutionary importance of adaptive phenotypic plasticity.

Natural systems typically contain diverse predator assemblages (i.e. more than two predator species) that include predators with different foraging techniques and locations and population densities. In addition, there could be other types of environmental variation that organisms encounter simultaneously with predation risk. Our challenges are to identify the environmental factors that are relevant for a particular organism, to determine how these factors may affect phenotypic responses, and to assess the fitness consequences of those responses. However, the traditional approach to addressing the adaptive value of inducible defenses has used simple experimental designs that include just the presence or absence of predators. While this is an excellent starting point, it overlooks the potential costs that inducible defenses may have in the multitude of other environments that prey encounter. For example, predator-induced phenotypes may have lower fitness with competitors (i.e. slower growth) or parasites (i.e. increased susceptibility), which may maintain inducible defenses versus constitutive defenses. In sum, studies that take a more comprehensive approach that includes the ecologically relevant selective environments for organisms will contribute substantially to our understanding of the ecology and evolution of phenotypic plasticity.

We also have a limited understanding of how prey respond to temporal variation in predation risk. During an individual's lifetime the environment may change state at any time in development (including reverting back to an earlier state). If alternative phenotypes are adaptive solutions to different environments, theory predicts that individuals that track environmental change will be favored by selection. Thus, prey that are able to track predation risk (i.e. produce defenses when predators are present and reduce defenses when predators are absent) should be favored by selection. However, such phenotypic flexibility may be constrained or limited (e.g., ontogenetic contingency, developmental constraints, unresponsive sensory systems) thereby decreasing the accuracy of matching the environment (Moran 1992, Padilla and Adolf 1996, DeWitt et al. 1998, Tollrian and Harvell 1999, Gabriel et al. 2005). The inability to respond to such fine-grained variation has major implications for whether phenotypic plasticity will be maintained in a population. In sum, the incorporation of natural patterns of temporal

environmental variation into our experiments will continue to provide valuable insights into the ecology and evolution of phenotypic plasticity.

Extensive attention has been devoted to understanding predator-induced plasticity from an ecological perspective. However, we have a considerably weaker understanding of predator-induced plasticity from an evolutionary perspective. Fortunately, a substantial body of theory exists to predict how phenotypes should evolve in response to environmental heterogeneity (Via and Lande 1985, Gavrillets 1986, Lively 1986, Via and Lande 1987, Via 1987, Van Tienderen 1991, Gomulkiewicz and Kirkpatrick 1992, Moran 1992, Van Tienderen and Koelewijn 1994, Van Tienderen 1997, Tufto 2000, Sultan and Spencer 2002). This theory predicts that populations should evolve specialized, non-plastic phenotypes in constant environments but generalized, plastic phenotypes in variable environments. When applied to predator-prey systems, the theory predicts that: 1) populations in constant predator environments should evolve highly-defended, non-plastic phenotypes, 2) populations in constant no-predator environments should evolve poorly-defended, non-plastic phenotypes, and 3) populations in variable predator/no-predator environments should evolve intermediate, plastic phenotypes. Freshwater snails provide an excellent system for testing these predictions because of their small body size, rapid generation times (2-3 generations/yr), and well documented responses to predators. Although such a long-term experiment is risky, I set out to test the above theoretical predictions in 2002. To date (after 5 years), the experiment has been extremely successful. Initial results from yearly plasticity assessments are encouraging but more data is needed before definitive conclusions are made about the experiment. Nonetheless, testing the evolutionary theory of plasticity evolution represents one of the most exciting steps in understanding the evolution of inducible defenses.

From a community perspective, phenotypic plasticity has dramatically changed our paradigm that changes in the density of species are the driving forces behind indirect interactions (DMIIs). Indeed, when a species alters its phenotype in different environments (e.g., predation, competition), this can alter how that species interacts with other members of the community. Such “trait-mediated indirect interactions” (TMIIs) can occur without a change in the density of the species (Abrams 1995). With the rising appreciation that TMIIs exist, new questions emerge. A major question is the relative contributions of DMIIs and TMIIs to species abundance and diversity. In recent reviews, it has been argued that TMIIs have been masked by DMIIs due to

the experimental designs used in previous studies (Werner and Peacor 2003, Schmitz et al. 2004). The key to unmasking the relative importance of TMIs versus DMIs is designing experiments that include the appropriate treatments. Including nonlethal predators (i.e. the predator is present but cannot reduce prey density) and thinning treatments (i.e. the predator is absent but prey density is reduced to simulate thinning by predators) helps to disentangle DMIs and TMIs. A few studies, explicitly designed to examine the relative contributions of DMIs and TMIs, have shown that although TMIs vary in magnitude they can be just as strong as DMIs (Huang and Sih 1991, Wissinger and McGrady 1993, Peacor and Werner 2001). These few studies illustrate the importance of TMIs relative to DMIs in several systems. However, additional studies that specifically address the relative importance of DMIs and TMIs are needed before we can make generalizations.

The above studies have taken the important first step of documenting that TMIs exist; however, these studies were conducted over relatively short time scales (i.e. less than a generation) preventing an assessment of the long-term importance of DMIs versus TMIs (Werner and Peacor 2003). For TMIs to play a role in structuring communities, they must alter the population parameters (e.g., r) of the species within the community. Thus, we need to conduct multi-generation, long-term studies that examine population parameters. Several theoretical models have taken this approach by specifically modeling trait plasticity (e.g. behavioral responses) and shown that it can influence population level responses and impact community structure (reviewed in Bolker et al. 2003, see also Abrams 1992, 1995, Krivan and Schmitz 2004). Unfortunately, many systems limit the researcher's ability to test these models due to long generation times, large body sizes, or the inability to manipulate predators over long time scales. To advance our understanding of indirect effects, studies must be conducted over several generations and include the necessary treatments to determine the relative importance of TMIs and DMIs.

As human population sizes increase, the impact that we have on ecological communities becomes greater. In particular, the world has increased the use of pesticides and the inevitable result has been the contamination of surrounding habitats. The growing use of pesticides on croplands, forests, and around homes brings to the forefront concerns about the effects pesticides have not only on target species, but also on non-target species of a community. To understand these effects, we need to apply a conceptual framework of density- and trait-mediated indirect

effects from the field of basic ecology (Relyea and Hoverman 2006). This framework can provide a more mechanistic understanding of the conditions under which pesticides affect species interactions, communities and ecosystems. Surprisingly, an ecological framework has only recently been applied to pesticide effects in communities. For my post-doctoral research, I will determine the utility of an ecological framework for predicting the effects of pesticides within aquatic communities.

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